ECOLOGICAL ROLES OF SOIL BIODIVERSITY

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"*An understanding of the natural world and what's in it is a source of not only a great curiosity, but great fulfillment*."

– David Attenborough

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General Introduction

Biodiversity

The biological diversity of the planet estimated to date is difficult to tangibly fathom with the known number of species being roughly 8.74 million (of which 74.7 % are terrestrial species), including around 298,000 plant species, 611,000 species of fungi**,** 36,400 species of protozoa and 10,100 classifiable prokaryotes, of which 96 % are bacteria (Mora *et al*. 2011a). Understanding the functional roles of such diversity and its evolutionary directions via the seemingly infinite possible interactions among these organisms and their surrounding environment is the crux of ecology. The vast majority of species in terrestrial ecosystems likely occurs belowground in soils, yet their functioning is relatively unexplored (Balvanera *et al*. 2006; van der Heijden *et al*. 2008). Soils can be highly diverse on small scales. For example, 10^{10}–10^{11} bacteria (Horner-Devine *et al*. 2003), 70,000 protozoa (Bonkowski 2004), and 200 m of fungal hyphae have been estimated to occur within a single gram of soil, as well as other meso- and macrofauna. The recent onset of high-throughput genetic sequencing of soil communities is revealing soil diversity levels termed 'hyper' diverse (Hibbett *et al*. 2009).

Importance of biodiversity

This vast overall diversity of organisms is understood to be the foundation in maintaining the productivity of ecosystem processes and the services societies depend upon, such as crop production and maintaining the fertility of soils (Hooper *et al*. 2005). For example, many studies have demonstrated greater plant species richness corresponds with improved ecosystem functioning, typically primary productivity, in experimental manipulations of grasslands (Tilman *et al*. 1996, Hector *et al*. 1999; Balvanera et al. 2006). However, such increased functioning in communities with greater diversity may not always be the case in natural environments, further illustrating the lack of understanding of the mechanisms behind biodiversity-ecosystem function relationships (Alder *et al*. 2011). Generally, biodiversity-productivity relationships can be attributed to differences in the functions and interactions among coexisting species, such that their overall effect in concert drives ecosystem processes and supports the services ecosystems provide society (Loreau & Hector 2001; Hooper *et al*. 2005). Additionally, the functioning of an ecosystem can be stabilized against environmental variations by greater species diversity providing 'insurance' that ecosystem processes are maintained through

natural and anthropogenic perturbations (Yahci & Loreau 1999; Tilman *et al*. 1998, 2006). For example, an environmental change inhibiting the functioning of some species may result in the reduction of that function at low levels of species richness, while in a more species rich ecosystem the functioning is maintained by other non-inhibited species (Loreau 2010).

However, there has been some debate surrounding a consensus as to the role and limits to which greater biodiversity improves ecosystem functions, the mechanisms behind biodiversity-ecosystem function relationships, and the overall importance of species diversity (eg. Huston 1997; Wardle 1999; and see Hillebrand & Matthiessen 2009 and Adler *et al*. 2011). Regardless, the limiting factor in understanding the driving forces behind biodiversity ecosystem function relationships arises from the inability to assess all interactions among species and the niches they occupy within a community. Nonetheless, empirical generalizations of how species function in a community, based on their functioning independently, have provided valuable insights into mechanisms behind biodiversity ecosystem functioning relationships.

The functioning of biodiversity

Under the null hypothesis, greater species richness does not alter the functioning of an ecosystem. This implies that species within a more rich community occupy an identical niche and their communal functioning is simply the average of their functioning independently (Loreau & Hector 2001; Fig 1a). However, as species are characteristically not identical, and thus utilize niche space differently, the addition of species requiring the same realized niche within a community will result in their competition and result in certain species being 'selected' against, thus limiting their contribution to a given ecosystem function (Fig. 1b; the 'selection effect'). On the other hand, species within a community that are not identical in niche requirements allows for greater coexistence, and thus for greater use of the available niche space and the multiple functioning of species that 'complement' each other within the community (Fig. 1c; the 'complementarity effect'). This reflects species performing better than expected based on their functioning independently. 'Selection' and 'complementarity' effects occur simultaneously under various scenarios within species communities and their combined effect is reflected in the overall functioning of an ecosystem (Loreau & Hector 2001; see Hector *et al*. 2002 for more detailed examples).

This analytical approach has provided insights for empirically assessing mechanisms behind biodiversity and its influence on ecosystems. For example, the classic positive asymptotic biodiversity – ecosystem function relationship can be explained by the presence of a few particularly effective species driving a species-rich community (the 'selection effect'), as well as niche differentiation and facilitative interactions (the 'complementarity effect'). In addition, the selection effect can be further partitioned into the dominance effect of a single species within a community (Fox 2005). All these mechanisms can drive relationships between increasing species richness and the functioning of an ecosystem, but with differing results. For example, increases in species richness that are redundant in function will result in stronger selection effects within the community and an asymptotic relationship between species richness and function (Fig. 1; Bell *et al*. 2005). This reflects the optimal level of ecosystem functioning being achieved at lower species richness, or once the probability of including the most functionally dominant species approaches 1; also termed the 'sampling / selection probability effect' (Huston 1997; Wardle 1999).

In the case of complementarity driven ecosystem functioning, increases in functioning with increases in species richness occur in a more linear fashion as additional species utilize more of the available niche space than communities with lower species richness (Fig. 1; Bell *et al*. 2005). The differentiation in the utilization of niche space among coexisting species is synonymous with functional dissimilarity and is the key component behind species diversity improving ecosystem functioning opposed to species richness *per se* (eg. Heemsbergen 2004, Jousset *et al*. 2011). Indeed, there are other possible species interactions resulting in negative effects of species richness, such as interference resulting from allelopathy and the trade-off between competitiveness and performing a function. For example, competition between mycorrhizal fungi for soil and host resources can alter their ability to promote host plant growth (eg. Bennett & Bever 2009; Becker *et al*. 2012). Nonetheless, positive effects of biodiversity are common (Balvanera *et al*. 2006).

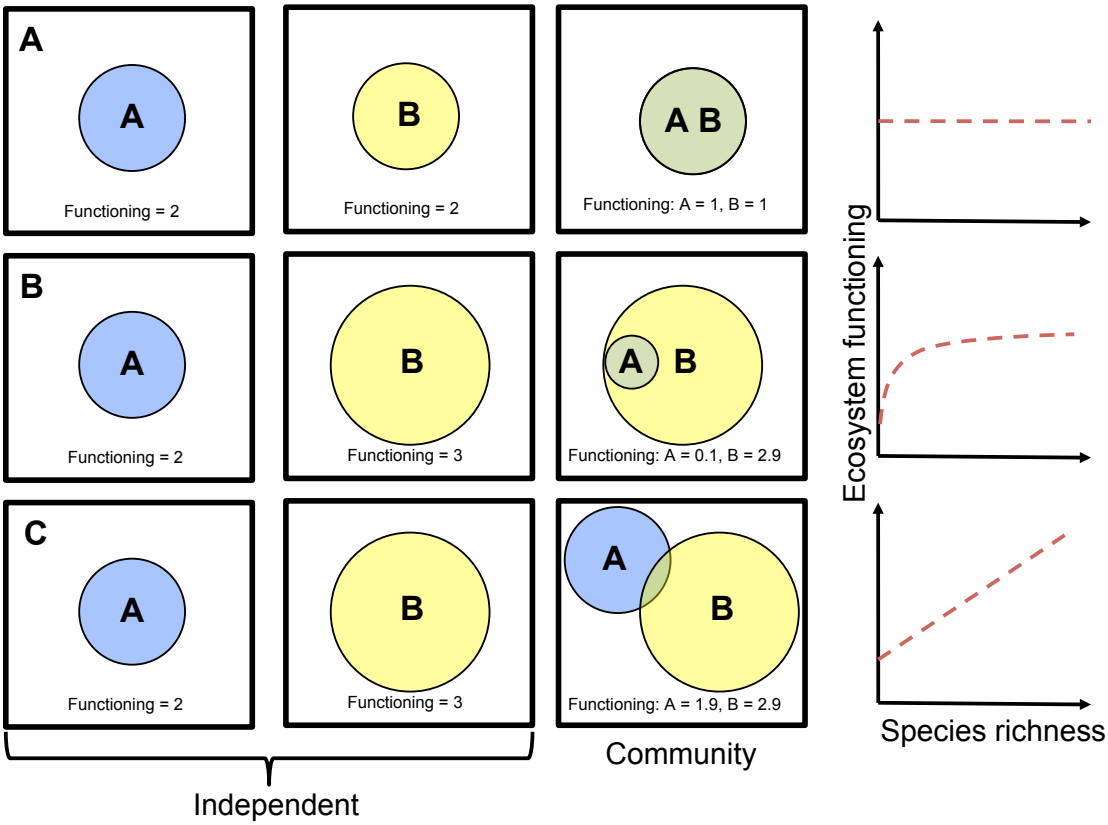

Figure 1. Examples of species (A and B) utilization of available niche space (represented by boxes) when functioning independently (fundamental niche) and within a community (realized niche). The hypothetical amount each species contributes to an ecosystem function (eg. plant biomass) is written within each box for each of these examples: (A) identical species (100% niche overlap) equally co-functioning within a community under the null hypothesis, (B) species differing in function, yet overlapping in niche space and their communal functioning driven by a strong selection effect, and (C) species differing in functioning with little niche overlap and utilizing more of the available niche space resulting in a strong complementarity effect. Relationships between species richness and ecosystem functioning under each of these scenarios are depicted by the dashed trend line on the right. The average of the two species functioning independently in these scenarios is the intercept in these relationships and the communal functioning of species under these scenarios is achieved at maximum ecosystem functioning in these examples.

Abiotic controls on biodiversity

Since the ability of more species rich communities to improve an ecosystem function depends upon their ability to function in a state of coexistence, the effects of more species rich communities is thus dependent upon the resources or niche space available within an environment. For example, increasing species richness within a hypothetical environment with only one resource in limited supply will only function as well as the species that are best able to utilize that sole resource. Increasing the availability of that sole resource may only enhance the ability of certain species to dominate. For example, the addition of nutrient fertilizers improves the productivity of plant species best able to utilize the nutrient addition and as a result are more

efficient in competing with neighbours for the next limiting resource (Hautier *et al*. 2009). Therefore, improving resource abundance may only alter the functioning of species communities to be driven by a selection effect.

In contrast to greater resources availability, resource heterogeneity should promote coexistence and thus complementarity in functioning of more species diverse communities (Hutchings *et al*. 2003; Ashton *et al*. 2010). Considering this, it is conceivable that the functioning of a species diverse community could shift between scenarios in presented in Fig. 1, depending on environmental conditions. An excellent example is provided by Jousset *et al*. (2011) demonstrating resource heterogeneity (complexity) as the environmental driver behind the improved functioning of more species rich communities. Thus, the functioning of a more biodiverse community is only as beneficial as the available niche space within the ecosystem.

Biotic controls on biodiversity

The functioning of a more species rich community may be further complicated by the multiple trophic interactions that occur in ecosystems. For example, through plant-soil feedback mechanisms soil communities mediate resource availability and moderate available niches within plant communities (Bever *et al*. 1997; Petermann *et al*. 2008) and vice versa; the presence of plant species, their density, and diversity mediate niches for soil organisms to function (Bezemer *et al*. 2010). Therefore, it is also important to consider not only the abiotic resource heterogeneity for maintaining biodiversity and its functioning, but also the biotic heterogeneity of interactions between trophic groups (Hooper *et al*. 2000; Duffy et al. 2007; Bastolla *et al*. 2009). This is a key feature of grassland ecosystems where plant antagonistic and mutualistic soil organisms influence the functioning of biodiversity in the aboveground plant community (eg. van der Heijden *et al.* 1998; Klironomos *et al*. 2000; Maron *et al*. 2011).

This intimate linkage between plant and soil communities is known to mitigate plant invasions (eg. Callaway *et al*., 2004; Reinhart & Callaway, 2006), plant community succession (van der Putten *et al*., 1993; Kardol *et al*., 2007), support primary productivity (eg. van der Heijden *et al*. 1998) and maintain plant diversity (Petermann *et al*., 2008). All of these processes also involve the 'Janzen-Connel' effect (Connel, 1971; Janzen, 1970). This process involves the build up of host specific predators/pathogens associating with the maternal plant resulting in a

reduction in the success of progeny in close proximity to the maternal plant. This mitigates species establishment and maintains diversity within the ecosystem. This concept has been refined in plant-soil community interactions where plants exerting an influence on the community structure of root endosymbionts can 'feedback' to influence the performance of the succeeding plant in a beneficial or antagonistic manner (Bever *et al*., 1997; van der Heijden & Horton 2009). This process not only helps to maintain diversity in natural ecosystems, but is also an integral part of agricultural systems and the basis behind crop rotations.

Biodiversity simplification and loss

Considering the importance of abiotic and biotic heterogeneity of ecosystems for the maintenance and functioning of ecosystems, the anthropogenic alteration and simplification of ecosystems has become a major concern. Increases in the human population have increased demands on the environment for food and other resources. This subsequently resulted in agricultural intensification and industrialization over the past century (Diraiappah & Naeem 2005; Mora *et al*. 2011b). These needs have been achieved with increased levels of chemical and energy inputs for the management of arable lands at the cost of declining species diversity (Matson *et al*. 1997; Krebs *et al*. 1999; Davies *et al*. 2006; Díaz *et al*. 2006; Rockström *et al*. 2009). This global issue holds potential repercussions for maintaining the planetary processes required for sustaining the current and expected human population (eg. Fig. 2; Matson *et al*. 1997; Krebs *et al*. 1999; Davies *et al*. 2006; Diaz *et al*. 2006; Rockström *et al*. 2009; Mora *et al*. 2011b). Current levels of biodiversity loss resulting from anthropogenic activities are thought to disrupt natural planetary process at levels exceeding what has been proposed as 'safe' to maintain the services ecosystems provide societies (Rockström *et al*. 2009; also see Fig. 2). This emphasizes the ever-increasing need for understanding not only whether greater biodiversity supports greater productivity in the services ecosystems provide, but also whether greater biodiversity is better able to maintain function and recover from environmental perturbations, both natural and human induced. This is particularly critical for soils considering agricultural intensification has marked effects ecosystem processes through the alteration of soil biotic communities and altered soil processes (Matson *et al*. 1997).

As in other aboveground ecosystems, anthropogenic activities have had adverse consequences for soil diversity resulting from land-use intensification and

increased nutrient availability (e.g. Helgason *et al*. 1998), nitrogen deposition (e.g. Lilleskov *et al*. 2002), and chemical contamination (Gans *et al*. 2005). The loss of species from an ecosystem can have cascading effects throughout the food web (Dunne *et al*. 2002). This may be of particular importance in soil ecosystems as the loss of species and simplification of trophic groups via anthropogenic activities can shift the soil fungal and bacterial community structure (Zeller *et al*. 2001; van der Wal *et al.* 2006) and reduce microbial diversity in soils (Giller 1996; Torsvik *et al*. 1996; Helgason *et al*. 1998; Gans *et al*. 2005). This likely has resounding implications for ecosystem functions as bacterial and fungal energy channels support different ecosystem processes (Wardle *et al*. 2004). For instance, richness of arbuscular mycorrhizal fungi (AMF) is often reduced under more intense agricultural management (Jansa *et al*. 2002; Oehl *et al*. 2004; Verbruggen *et al*. 2010). These fungi are well known to provide soil resources to many plant species, including crops, and their suppression and loss from soils may have consequences for plant productivity (Smith & Read 2008).

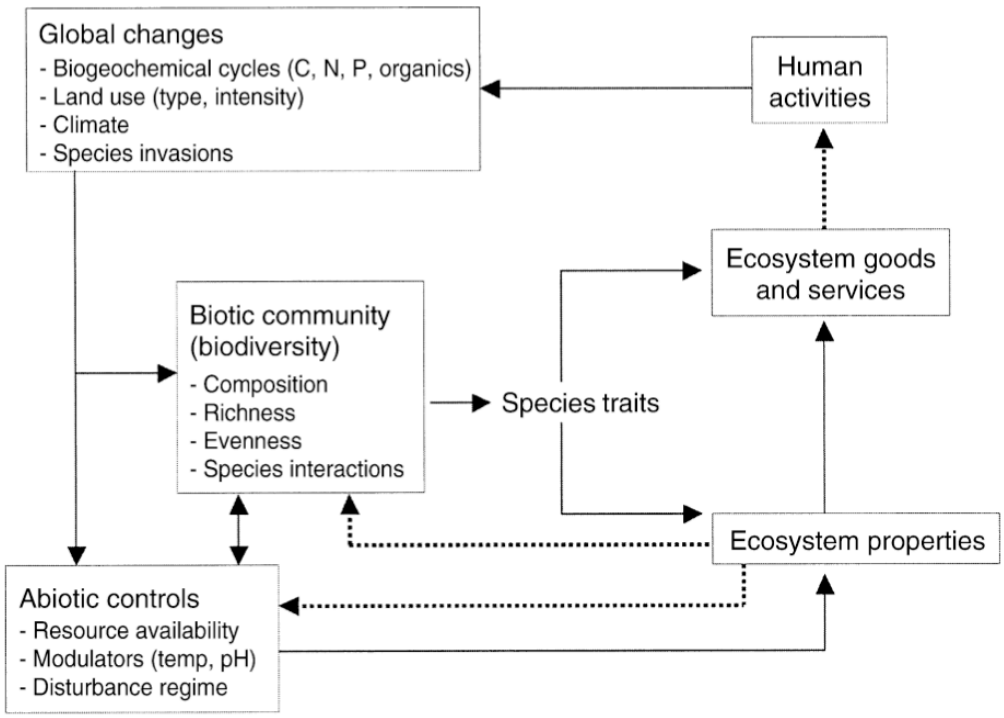

Figure 2. Schematic of the interactions that link the effects of human activities on global changes to the ability of ecosystems to provide services upon which human societies depend (taken from Hooper *et al*. 2005). Human activities, from greenhouse gas emissions to agricultural management, alter the abiotic characteristics of ecosystems, such as resource availability and heterogeneity. This also holds direct and indirect consequences on species ability to coexist and may lead to species loss and invasions. This alters the contribution of individual species to an ecosystem function and thus the functioning of the overall of the biotic community is altered (summed up in species traits). This in turn alters the effect that the biotic community has on the properties of the ecosystem and importantly the sustainability of ecosystem processes and the goods it produces on which human societies depend. Such changes in the productivity of an ecosystem alter human activity (eg. poor crop production results in agriculture intensification) and the cycle continues.

Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are obligatory root endophytes consisting of roughly 300 known species forming the phyla Glomeromycota, with a cosmopolitan distribution (Opik *et al*. 2010; see Fig. 3a for a sample of the variety of AMF found within a soil sample). Moreover, these plant root-inhabiting fungi have been observed in ancient fossilized plants and are thought to be a key component in the evolution of land plants (Brundrett *et al*. 2002). These fungi associate with a majority of terrestrial plant species and commonly engage in symbiotic relationships with their plant hosts in order to acquire photosyntheticlly derived carbon (Smith & Read 2008; illustrated in Fig. 3b). Plants frequently benefit from this association by acquiring soil resources otherwise inaccessible to the plant host; typically P, but increased uptake of other nutrients and minerals are known to occur (Marshner & Dell 1994; Hodge *et al*. 2001). This often results in observable benefits to plant productivity, the ability for plants to coexist (eg. Scheublin *et al*. 2007; Wagg *et al*. 2011a; see Fig. 3c) and defend against predators and pathogens (eg. Gange & West 1994; Newsham *et al*. 1995; Azcón-Aguilar & Barea 1996). It should be noted that negative effects of AMF on plant hosts also occurs (eg. Francis & Read 1995; Rinaudo *et al*. 2010; Veiga *et al*. 2011). However, the mechanisms underlying this effect are unclear, although many have been suggested and debated (see Li *et al*. 2008; Facelli *et al*. 2009; Kaschuk *et al*. 2009). Moreover, these scenarios are likely context dependant on host and abiotic soil properties (Johnson 2010; Hoeksema *et al*. 2010).

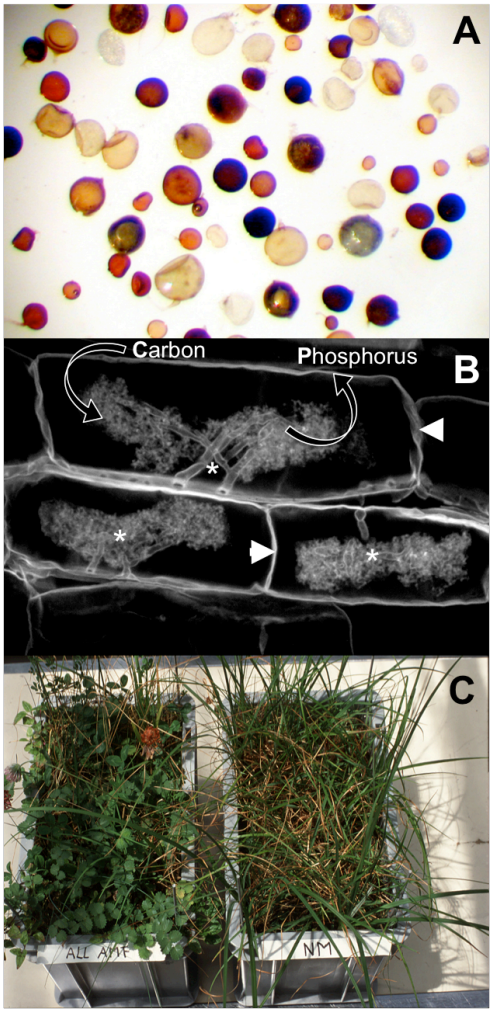

Figure 3. Characteristics of arbuscular mycorhizal fungi. (A) Spores of a mixture of AMF fungi isolated from field soil. The various colours and sizes indicate the diversity (from Peterson *et al*. 2006). (B) The exchange interface between plant host and fungus occurring within the inner cortex of the host root. Highly branched arbuscule structures (indicated by astrics) specialized for maximizing surface to area ratio between plant and fungus for the exchange of photosynthetic carbon for soil nutrients (plant cell walls indicated by arrowheads; image modified from Peterson *et al*. 2006). (C) The effect of the presence of AMF on plant community diversity (shown on the left), is clearly visible in contrast to the plant community diversity in the absence of AMF on the right (from van der Heijden *et al*. 2006).

Functioning of AMF communities

These plant-fungal associations have been demonstrated to be a key component of the soil biota as their presence can strongly influence plant productivity, plant diversity and plant community composition (van der Heijden *et al*. 1998). Furthermore, a greater richness of AMF taxa has been associated with greater host community diversity and productivity (van der Heijden *et al*. 1998; Maherali & Klironomos 2007; Wagg et al. 2011b). However, other studies have observed the single most productive AMF monoculture can have a similar effect as AMF mixtures (Vogelsang *et al.* 2006; Jansa *et al*. 2008). It has been suggested that

the relationships between AMF richness and plant productivity may then simply result from a sampling probability effect, whereby the likelihood of including the fungal species with the strongest effect on functioning increases with AMF richness. As in grassland biodiversity projects this has questioned the importance of AMF diversity versus the presence of a keystone species in achieving maximum benefits from more AMF rich communities (Wardle 1999; van der Heijden *et al*. 1999); which further underlines the lack of current knowledge in how a more diverse AMF community functions to improve plant productivity.

Nonetheless, the identity and richness of the AMF present within an AMF community both contribute to observed plant community characteristics. This is perhaps a result of the imbalance in AMF-derived benefits received between competing host plants, as not all AMF taxa equally benefit the hosts with which they associate (Ravenskov & Jakobsen 1995; Klironomos 2003). Due to this variation in host specific benefits, AMF communities could therefore ease belowground competitive interactions between plants by improving accessibility of the plant community to the soil resource pool. Previously it has been demonstrated that the presence of AMF mediate the competitive balance between sympatric plants via resource partitioning among associated hosts (Hartnett *et al*. 1993; Zobel & Moora 1995; Scheublin *et al*. 2007), but the effect of their diversity is generally unknown.

Mechanisms affecting AMF community function

Host specificity and preference play an important ecological and evolutionary role and are commonly observed in plant communities (Janzen 1970; Connell 1971; Reynolds *et al*. 2003). Previous studies have shown that the pairing of plant host - AMF taxa compatibility is largely promiscuous in greenhouse studies and lacks absolute fungus-plant taxa specificity (Smith & Read 2008). However, in field situations fugal preference toward a particular host plant can occur (Sanders 2003; Croll *et al*. 2008). This implies host preference as a mode of reducing niche overlap and improving coexistence in AMF communities, thus allowing for greater complemenarity in their communal function. The functional role of niche partitioning among AMF has been observed to occur via spatial separation (Bever *et al*. 2009), which could manifest via host preference in a plant polycultures. The complementary functioning of AMF communities could therefore be mediated by a trade-off between AMF-AMF competitive abilities and host growth promotion (Bennett & Bever 2009). Additionally, the niche dissimilarity in AMF taxa not only includes host preferences

(Bever *et al*. 2001, Vandenkoornhuyse *et al*. 2003), but also pathogen protection (Wehner *et al.* 2010) and nutrient uptake abilities (Jansa *et al*. 2005; Thonar *et al*. 2011); all of which could potentially function in concert in a complementary manner to enhance overall plant community productivity and diversity (Koide *et al*. 2000; Rillig 2004). However, such niche partitioning within AMF communities and the resulting effects on ecosystem functioning remains untested.

The abiotic conditions of the soil also have a strong influence on how AMF communities function and are assembled. For example, different AMF taxa can dominate under particular environmental conditions, such as in different soil types (Lekberg *et al*. 2007; Oehl *et al*. 2010). However, at a localized scale, within a single soil type, the community assembly of AMF appears to be stochastic (Sorensen & Rosendahl 2011). In addition, the effects of AMF communities on plant hosts may be suppressed and even antagonistic with increased levels of plant available nutrients, such as with the application of chemical fertilizers that reduce the dependency of plants on AMF for nutrient acquisition (Johnson 1993; Johnson 2010). Recently, a meta-analysis by Hoeksema *et al*. (2010) of AMF effects on plant hosts revealed a strong context dependence on abiotic and biotic conditions. In general, AMF-plant associations are more beneficial for the host depending on the functional group of the host as well as the availability of N and P in the soil (Hoeksema *et al*. 2010). This emphasizes the need for addressing the functioning of AMF-plant communities in multiple contexts for discerning general trends. As a result, the mechanisms by which more diverse AMF communities are governed that influence ecosystem functioning are largely elusive. Nonetheless, the ability to culture these fungi and manipulate their diversity within the soil in controlled conditions and the well studied effects on plant hosts provides an excellent model system for addressing the mechanisms behind the functioning of biodiversity, both within and between the two above and belowground trophic groups.

Thesis outlook

I set out to assess the functioning of AMF diversity beginning with assessing the effects of their diversity and identity on plant coexistence; the key mechanism behind the complementarity effect in species richness – productivity relationships (Chapter 1). This is followed by assessing the collective contribution of interactions among AMF to plant productivity along an increasing continuum of AMF richness (Chapter 2). Since AMF-plant interactions are the mechanism by which species rich

communities function can depend on abiotic environmental conditions, both these research objectives were addressed in two contrasting soil conditions. To further assess how individual AMF function within a community to collectively improve plant community productivity, I explore the possibility of niche differentiation among AMF taxa via host and substrate preference as a mechanism by which AMF communally improve plant productivity (Chapter 3).

Although, AMF and their diversity can have considerable influence on plant community characteristics, they are only a fraction of the biodiversity in soil communities. As a result, I address the consequences of soil biodiversity loss on the functioning of a grassland plant community and soil processes. This is an on-going project and the most current data available are presented to illustrate the negative consequences of soil biodiversity loss on multiple ecosystem functions (Chapter 4).

In an effort to extend current knowledge of the ecological importance of soil biota, I assess the influence of soil feedback effects on intraspecific heritable differences in the agriculturally important, and soil symbiont dependant, *Trifolium pratense* to demonstrate the potential of soil biota to influence local adaptation in plants (Chapter 5). This study is aimed at opening new avenues for soil microbial research in the hope of bridging the evolution of plant populations and soil ecology, as the two are tightly linked (Hoeksema 2010).

The data presented within this dissertation aids in shedding light into the functioning and ecological importance of the unseen and complex world belowground. There is still much need for a deeper understanding of the mechanisms that influence soil biodiversity – ecosystem functioning and the many trophic interactions within soils that are required to maintain the services ecosystems provide society under future climatic and environmental changes. This knowledge is crucial for the maintaining productivity and sustainability both natural and agricultural systems.

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Chapter 1

MYCORRHIZAL FUNGAL IDENTITY AND DIVERSITY RELAXES PLANT-PLANT COMPETITION

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ABSTRACT

There is a great interest in ecology to understand the role of soil microbial diversity for plant productivity and coexistence. Recent research has shown increases in species richness of mutualistic soil fungi, the arbuscular mycorrhizal fungi (AMF), to be related to increases in aboveground productivity of plant communities. However, the impact of AMF richness on plant–plant interactions has not been determined. Moreover, it is unknown whether species-rich AMF communities can act as insurance to maintain productivity in a fluctuating environment (e.g. upon changing soil conditions). We tested the impact of four different AMF taxa and of AMF diversity (no AMF, single AMF taxa and all four together) on competitive interactions between the legume *Trifolium pratense* and the grass *Lolium multiflorum* grown under two different soil conditions of low and high sand contents. We hypothesized that more diverse mutualistic interactions (e.g. when four AMF taxa are present) can ease competitive effects between plants, increase plant growth and maintain plant productivity across different soil environments. We used quantitative PCR to verify that AMF taxa inoculated at the beginning of the experiment were still present at the end. The presence of AMF reduced the competitive inequality between the two plant species by reducing the growth suppression of the legume by the grass. High AMF richness enhanced the combined biomass production of the two plant species and the yield of the legume, particularly in the more productive soil with low sand content. In the less productive (high sand content) soil, the single most effective AMF had an equally beneficial effect on plant productivity as the mixture of four AMF. Since contributions of single AMF to plant productivity varied between both soils, higher AMF richness would be required to maintain plant productivity in heterogeneous environments. Overall this work shows that AMF diversity promotes plant productivity and that AMF diversity can act as an insurance to sustain plant productivity upon changing environmental conditions.

INTRODUCTION

There is currently great interest in understanding the role of species richness and diversity in regulating ecosystem processes (Hooper et al. 2005). The relationship between plant diversity and plant productivity has already received much attention (e.g. Tilman et al. 1996, Hector et al. 1999, Loreau et al. 2002); however, the significance of soil microbial diversity for aboveground plant productivity is still poorly understood (Balvanera et al. 2006). Few studies have investigated whether soil microbial diversity can influence plant community productivity and plant–plant interactions (see Rillig 2004; van der Heijden et al. 2008). Here we focus on arbuscular mycorrhizal fungi (AMF), a group of obligatory root endophytes that form mutualistic associations with the majority of land plants by improving nutrient uptake in plant hosts (Smith and Read 2008).

Recently, Bastolla et al. (2009) illustrated how an increased number of mutualistic interactions can relax competition in species networks and thus increase biodiversity. In a similar manner, greater diversity of AMF taxa may ease belowground competitive interactions between plants as AMF taxa can have specific host preferences (Bever et al. 2001, Vandenkoornhuyse et al. 2003) and differ in functional compatibility between host plants (Ravnskov and Jakobsen 1995). Moreover, the variation in host benefits provided by differing AMF taxa, such as improved pathogen resistance (Newsham et al. 1995*b*, Maherali and Klironomos 2007) and nutrient uptake abilities (Jansa et al. 2005) may enhance plant species complementarity and thus overall plant productivity (Koide et al. 2000; Rillig 2004).

There have been a handful of studies that have begun to address the importance of AMF diversity for the productivity of a plant community (van der Heijden et al. 1998*a*, van der Heijden et al. 2003, Balvanera et al. 2006, Vogelsang et al. 2006, Jansa et al. 2008). Some studies show AMF diversity can enhance the productivity of single plant species (Lekberg et al. 2007, Maherali and Klironomos 2007) or a community of grassland plants (van der Heijden et al. 1998*a*). Other studies found that a particular AMF taxon can be as beneficial or even more beneficial to plant growth than the mixture of several AMF (van der Heijden et al. 1998*ab*, van der Heijden et al. 2003, Vogelsang et al. 2006, Jansa et al. 2008), suggesting relationships between AMF diversity and plant productivity to be mainly due to a sampling probability effect (Wardle 1999). However, it has yet to be demonstrated how AMF diversity may affect plant competition and plant complementarity effects, i.e. the other major mechanism (Loreau and Hector 2001,

Cardinale et al. 2007) underlying positive plant biodiversity effects on plant productivity.

Studies investigating whether AMF can alter competitive interactions have mainly concentrated on comparisons of AMF taxa (Scheublin et al. 2007) or the absence versus presence of AMF inoculum on plant competition (Fitter 1977, Hartnett et al. 1993, Hetrick et al. 1994, Zobel and Moora 1995, Schroeder-Moreno and Janos 2008, Collins and Foster 2009). It is conceivable that the wide range of AMF–host plant interactions may reduce the overlap of resource niches among coexisting plants, thus reducing interspecific competition and increasing complementarity between host plants and potentially increasing total plant community productivity.

Most ecosystems harbour a diverse AMF community in the soil (Bever et al. 2001). Different AMF taxa can dominate under particular environmental conditions, such as in different soil types (Oehl et al. 2010). Hence, the functioning of specific AMF taxa may be depressed in one soil type and enhanced in another, yet the influence of the AMF community on aboveground productivity in different soil types would be maintained. This “insurance hypothesis” (Yachi and Loreau 1999) of AMF richness for the maintenance of plant coexistence and productivity in heterogeneous soil environments has yet to be tested.

In this study, the effects of AMF species richness and soil conditions on competitive interactions between a grass (*Lolium multiflorum* Lam.) and a legume (*Trifolium pratense* L.) were tested. We chose a grass-clover plant community as model system as these two species commonly coexist in agricultural and natural grassland ecosystems (Nyfeler et al. 2009), and because grasses and clover respond differently to AMF, with clover usually benefitting from AMF and many grasses being unresponsive (van der Heijden et al. 1998; Scheublin et al. 2007). We hypothesize that (1) co-inoculation of four AMF taxa improves the co-existence between two competing host plant species compared to inoculation of single AMFs and (2) the influence of AMF, inoculated individually and as a community, on competitive interactions between plants differs depending on the soil conditions. To test these hypotheses we grew *T. pratense* and *L. multiflorum* in a replacement series in the absence of AMF (AMF richness = 0), inoculated with one of four AMF taxa individually (low AMF diversity: richness = 1) and the combination of all four AMF taxa (high AMF diversity: richness = 4). This model system was replicated in two different soil-sand mixtures to demonstrate the role soil conditions can play in

mediating AMF community effects on plant-plant competitive outcomes. The four AMF taxa originated from Swiss grasslands where they commonly co-occur. We used quantitative PCR to confirm the presence of the AMF used as inocula at the end of the experiment in the plant roots to provide confirmation of the co-colonization of roots by all four AMF within the high diversity treatment.

METHODS

Soil and inoculum preparation

Field soil was collected from a long-term grassland field harboring native *Lolium* and *Trifolium* species located at the Agroscope Reckenholz research station in Zürich, Switzerland (047° 25' 38.71'' N, 8° 31' 3.91'' E). Collected soil was then sieved through a 1 cm mesh in order to remove large stones and root fragments. This soil was mixed with sand by volume in the ratios of 1:4 and 4:1 soil to sand to create two different soils with a "high sand" and "low sand" content, respectively. The two soil-sand mixtures were autoclaved for 99 min at 121 °C. Two samples of approximately 1 kg from each soil type were taken for nutrient analysis after autoclaving.

The high sand soil had a pH of 7.7 with 0.1 % organic C, 2.45 % clay, 8.2 % silt and 89.2 % sand and contained 20.5 $mg \cdot kg^{-1}$ of water soluble inorganic N (NO3– and NH4+) determined with a Skalar segment flow analyzer. Plant available P_2O_5 and K_2O, extracted by CO_2-saturated water, was 0.71 $mg \cdot kg^{-1}$ and 5.0 $mg \cdot kg^{-1}$ respectively. The ammonium acetate-EDTA (pH 4.65) extracted amounts of Ca, P, K and Mg in $mg \cdot kg^{-1}$ were 7.02×10^3, 33.5, 2.85 and 96.6, respectively. The low sand soil had a pH of 7.5 with 0.9 % organic C, 12.2 % clay, 20.8 % silt and 64.4 % sand and contained 50.5 $mg \cdot kg^{-1}$ of water-soluble inorganic N. Plant available P_2O_5 and K_2O was 0.32 $mg \cdot kg^{-1}$ and 7.5 $mg \cdot kg^{-1}$ respectively. The ammonium acid-extracted Ca, P, K and Mg in $mg \cdot kg^{-1}$ was 4.26×10^3, 17.7, 24.4, and 160.9, respectively.

The four AMF fungi used were: *Glomus mosseae* (isolate BEG161, Jansa et al. 2002), *G. intraradices* (BEG 21, van der Heijden et al. 2006), *G. claroideum* (isolate JJ132, Jansa et al. 2002) and *Diversispora celata* (FACE 234, Gamper et al. 2009). These four AMF belong to the family Glomeraceae, each representing a specific clade (*Glomus* group Aa, Ab, B and C, respectively) and are common in Swiss arable and grassland soils (Schüssler et al. 2001, Schwarzott et al 2001, Gamper et al. 2009, Oehl pers. com.). These fungi were cultured on *Plantago lanceloata* L. in pots of 1 L volume for 5 months. The substrate was sand mixed with

approximately 15 % field soil, receiving 20 ml Hoagland's nutrient solution (Hoagland and Arnon 1950) with ¼ original concentration KH_2PO_4 every two weeks and watered to maintain 20 % soil moisture by weight. A control inoculum (no AMF) was prepared in the same way as the four AMF inoculants. *Glomus claroideum, G. intraradices*, *G. mosseae* and *D. celata* inoculants were observed colonizing 30.4 %, 90.5 %, 42.1 % and 17.5 % of the root length of *P. lanceolata* with 22, 38, 2 and 121 spores per cm^3 of soil, respectively. No AMF spores or colonization of roots were observed in the control inoculum.

Preparation of AMF treatments and plant seedlings

For the experiment, the cultured AMF material was transferred to 1 L pots containing 1.15 kg (dry weight) of one of the two soil-sand mixtures, high sand or low sand. Pots inoculated with a single AMF received 50 ml of inoculum, containing substrate and root fragments, of one of the four AMF. Treatments co-inoculated with all four AMF species received 12–14 ml of roots and substrate of each AMF inoculum; totaling again 50 ml of inoculum. The inocula were mixed throughout the soil substrate within each pot.

Seeds of *Trifolium pratense* var. Milvus and *Lolium multiflorum* var. Daxus, originating from seed multiplication plots located at the Agroscope Reckenholz research station in Zürich, were surface sterilized by agitating them in 1.25 % sodium hypochlorite (diluted household bleach) for 5 minutes followed by a thorough rinse in dH_2O. The seeds were then allowed to germinate on 1.5 % water agar during 2–4 days. Seedlings were then transplanted evenly spaced into the AMF-inoculated pots. Pots were covered with cellophane for three days to allow seedlings to establish. Seedlings that did not survive were replaced up to two weeks after initial planting.

A microbial wash was created by using 1 L of the same un-autoclaved field soil used to create the two soil treatments and wet sieving it through a series of sieves with the smallest being 11 μm with 5 L of dH_2O. Ten ml of this was added to each pot after planting in order to standardize the microbial community within each pot with a natural grass/clover soil microbial community including rhizobia bacteria (evidenced by numerous root nodules on red clover).

Pots were randomly distributed in two adjacent greenhouses. Plants were allowed to grow for 25 weeks with 16 h / 25 °C days and 8 h / 16 °C nights. Plants received natural light and supplemental illumination was provided by 400-W high-

pressure sodium lamps to maintain a light level above 300 W/m^2. Pots were watered with dH_2O by weight as required to maintain soil moisture in the range of 10–20 %.

Data collection

Shoots were harvested 9, 16 and 25 weeks after planting in order to reduce aboveground competition for light and to simulate mowing/grazing as is usually done in managed grass-clover meadows/pastures. At the time of harvest at 9 and 16 weeks shoots were cut at approximately 5 cm above the soil surface. During the final harvest at 25 weeks shoots were cut directly at the soil surface and roots were rinsed clean of soil and frozen at –20 °C until they could be processed further. Shoots were dried at 80 °C after each harvest and the biomass recorded to the nearest tenth of a mg. The aboveground biomass of each plant species was pooled across harvests and used in all subsequent analyses.

Frozen roots were thawed, cut into 1–2 cm fragments, and mixed for molecular assessment of AMF (see below) and for determining AMF root colonization. To determine the level of colonization of each single-AMF inoculated treatment and for assessing the viability of the inoculants, a random sample of 1–2 g of fresh root was fixed in 50 % ethanol overnight, cleared with 10 % KOH in an 80 °C water bath for 45 min and then stained with 5 % pen-ink vinegar (Vierheilig et al. 1998) for 10 min in an 80 °C water bath. A random selection of the cleared and stained roots were mounted on glass slides with 50 % glycerine under a cover slip and scored for the presence AMF using the intersect method (McGonigle et al. 1990) for 100 intersects.

The presence of the four different AMF in the high-diversity AMF treatment was determined using quantitative real-time PCR (qPCR) with hydrolysis probes targeting species-specific motifs of the large ribosomal subunit (LSU) of *G. mosseae*, *G. intraradices* and *G. claroideum,* following the protocol developed by Thonar (2009); see Appendix A for details. For the qPCR quantification of *D. celata*, novel primers and a hydrolysis probe were designed (see Appendix A). The primers for *D. celata* also targeted a fraction of the LSU ribosomal gene copies, similar to the three other AMF taxa.

Experimental design and statistical analyses

The experiment was set up as a randomised block design with two blocks (replicates evenly divided between 2 greenhouses), two soils (high sand and low

sand) and six AMF treatments (no mycorrhiza, AMF I, AMF II, AMF III, AMF IV and AMF I+II+III+IV). The twelve combinations of two soils × six AMF treatments were factorially combined with five plant-competition treatments. These reflected a replacement series between *T. pratense* and *L. multiflorum*, i.e. individuals of the two species were planted in the following ratios: 8:0, 6:2, 4:4, 2:6, or 0:8. Each treatment combination was replicated six times for a total of 360 pots.

Two pots were found to be contaminated with AMF not initially inoculated into the pots by both light microscopy and real-time PCR. These two pots were removed from the data set. One pot, with 6 *T. pratense* and 2 *L.multiflorum* in high sand soil, initially non-mycorrhizal, was found to be colonized by *G. claroideum*. The second pot of 8 *T. pratense* in low sand soil, which was initially inoculated with *D. celata*, was contaminated with *G. intraradices*.

The aboveground biomass of *T. pratense* and *L. multiflorum* was assessed with a three-way analysis of variance (ANOVA) with soil, planting ratio, AMF treatment and their interactions as main sources of variation. One-way ANOVAs and Tukey HSD tests were used to further assess the variation in aboveground biomass among AMF treatments within each soil × planting ratio. The two greenhouses in which plants were grown was used as a block effect in all ANOVAs.

Competitive interactions between the two plant species were determined by assessing the growth per individual plant within a mixture relative to that in monoculture; which was calculated as the relative yield per individual (RY_{ind}) by the equation: $RY_{\text{ind}} = \frac{O_{ij}}{M_{ij}}$.

Here O_{ij} is the observed aboveground biomass per individual of plant species *i* grown in mixture within a pot of a soil × AMF treatment combination *j* and M_{ij} is the mean aboveground biomass per individual within the monoculture of plant species *i* present within a pot of the same soil × AMF treatment combination *j* (de Wit 1960). The relative yield per individual (RY_{ind}) portrays the mean change in shoot biomass production of an individual plant as conspecifics are replaced by heterospecifics under the same planting density.

In addition, the relative yield per stand (RY) was also calculated from the observed aboveground biomass per species in mixture divided by the aboveground biomass per species in monoculture. The relative yields per stand of the two plant species in mixtures were added to obtain relative yield totals (RYTs) for each soil × AMF treatment combination (de Wit et al. 1966). The RYT provides an overall

summary of changes in the total aboveground biomass in mixtures relative to monocultures and is often used to assess overyielding in grass-clover mixtures, where values greater than 1 indicate a greater biomass production in mixtures than the average of the two plant species in monoculture (see Weiner 1980, Kirwan et al. 2007, Marquard et al. 2009).

A three-way ANOVA was used to test the effects of AMF treatment, soil and planting ratio, as well as their interactions, on the RY_{ind} of *T. pratense* and *L. multiflorum* and on RYT in mixtures, with the greenhouse in which the plants were grown added as a block effect. The RY_{ind} and RYT were assessed for differences from 1 (RY_{ind} = 1 and RYT = 1, respectively) within each soil × AMF treatment combination in order to determine the influence of each AMF on the competitive interactions between the two host species as well as their influence on overyielding in plant mixtures. The effect of each AMF on the RY_{ind} of *T. pratense* and *L. multiflorum* and the RYT was also assessed using contrasts to determine differences in individually inoculated AMF treatments from the non-mycorrhizal control as well as the high-diversity AMF treatment with all AMF co-inoculated.

In order to improve homoscedasticity in the data, Box-Cox transformations were used for the assessment of aboveground biomass and relative yield measures were log transformed prior to analyses. Means were considered to differ significantly at a type-I error level of $\alpha < 0.05$. Statistical analyses were carried out using R 2.10.1 (R Foundation for Statistical Computing 2009).

RESULTS

Aboveground biomass

Overall the total shoot biomass of *T. pratense* was strongly influenced by planting ratio and AMF treatment, but not by soil conditions (Table 1). The total biomass of *T. pratense* was greater in mixtures where it was more abundant (Fig. 1A and B). Inoculation with AMF increased *T. pratense* biomass up to 15 times compared to the non-mycorrhizal treatment depending on soil and planting ratio (Fig. 1A and B), resulting in a significant three-way interaction effect (Table 1). In all planting ratios within the high sand soil, the greatest *T. pratense* biomass occurred in the inoculation treatments with *D. celata* or with all four AMF; with *D. celata* frequently being the greater of the two, followed by the inoculation treatments with *G. intraradices*, *G. claroideum* or *G. mosseae*, which were generally similar in effect (Fig. 1A). Within the low sand soil, the high-diversity AMF, *D. celata* and *G.*

claroideum treatments had large beneficial effects on *T. pratense* biomass and were commonly similar in effect; however, in mixtures with *L. multiflorum*, inoculation with all four AMF consistently yielded the greatest *T. pratense* biomass (Fig. 1B).

The shoot biomass of *L. multiflorum* was most strongly influenced by soil conditions, followed by AMF treatment and planting ratio (Table 1; Fig. 1C and D). Shoot biomass of *L. multiflorum* was greatest in the low sand soil as well as in mixtures in which it occurred in high proportion (Fig. 1D). However, unlike *T. pratense*, the *L. multiflorum* biomass did not vary consistently among AMF treatments and was generally greatest in the non-mycorrhizal treatment (Fig. 1C and D). In no case was the shoot biomass of *L. multiflorum* significantly improved by the presence of AMF (Fig. 1C and D). Whether AMF inoculation resulted in a significant depression in *L. multiflorum* biomass depended on soil conditions and planting ratio (Fig. 1C and D).

Table 1. Results of the ANOVA testing for the effects of soil conditions (Soil), planting ratio (Ratio) and AMF treatment (AMF) on the overall aboveground biomass of *L. multiflorum* and *T. pratense*. Prior to analyses Box-Cox transformations were used to improve the homoscedasticity of the residuals. The greenhouse in which plants were grown is represented by the 'Block' effect.

		T. pratense		*L. multiflorum*	
Source of variation	*df*	F	p	F	p
Block	1	1.14	0.29	2.44	0.12
Soil	1	0.93	0.34	5.29×10^3	< 0.0001
Planting Ratio	3	1.78×10^3	< 0.0001	11.2	< 0.0001
AMF	5	327	< 0.0001	14.5	< 0.0001
Soil x Ratio	3	136	< 0.0001	3.45	0.02
Soil x AMF	5	13.5	< 0.0001	3.31	0.007
Ratio x AMF	15	8.03	< 0.0001	3.50	< 0.0001
Soil x Ratio x AMF	15	2.49	0.002	0.96	0.49
Error	237				

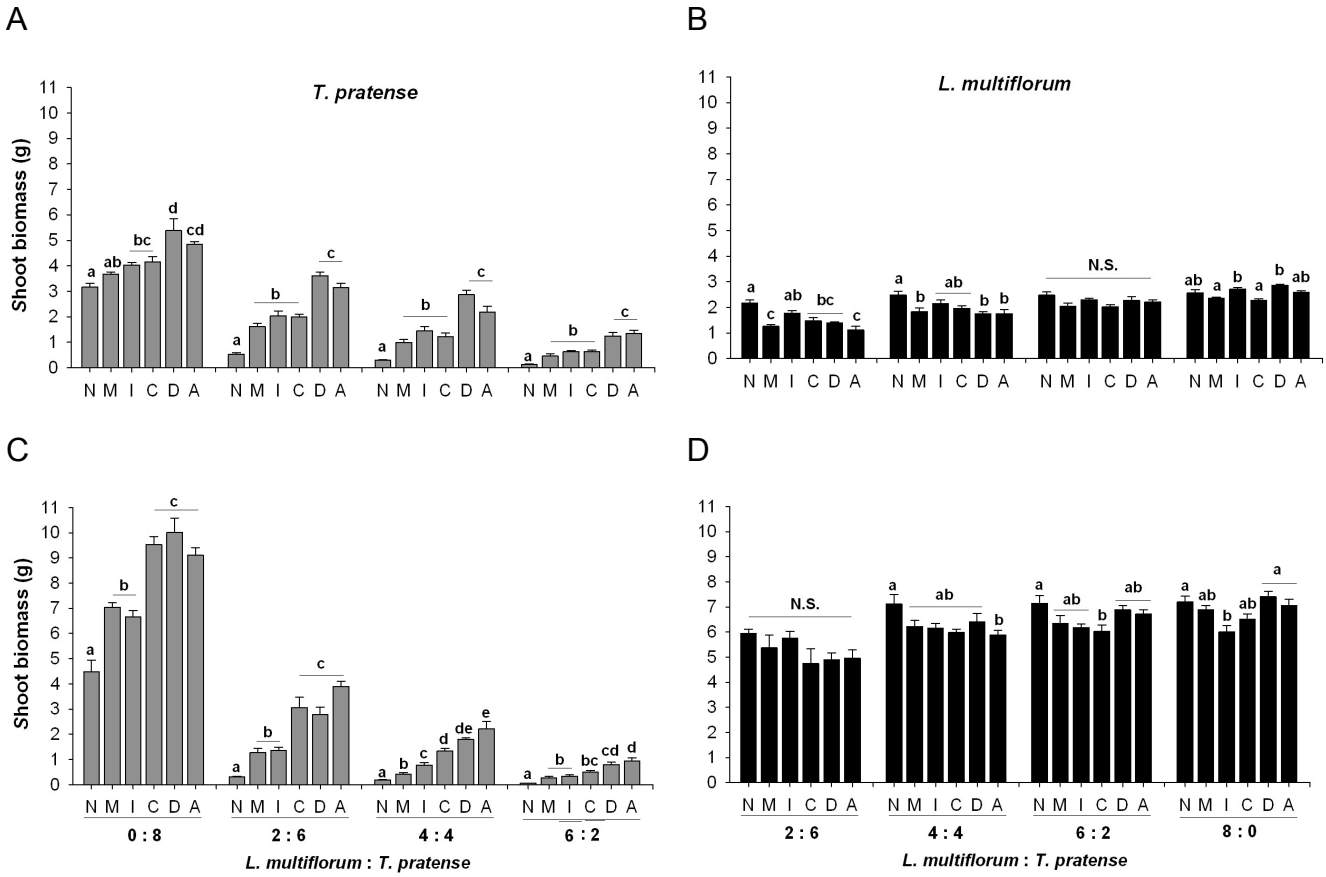

Figure. 1. Mean and standard error (SE) of the aboveground biomass of *T. pratense* (grey bars) and *L. multiflorum* (black bars) are shown for each planting ratio (*T. pratense* : *L. multiflorum*) and AMF treatment combination in both high sand (A and B) and low sand (C and D) soils. AMF treatments are denoted as: N = non-mycorrhizal, M *= G. mosseae*, I = *G. intraradices*, C = *G. claroideum*, D = *D. celata*, A = inoculation with all 4 AMF taxa. Significant differences (Tukey HSD $p > 0.05$) between AMF treatments within each planting ratio and soil are indicated by different letters. N.S. = not significant.

Relative yields

The relative yield per individual (RY_{ind}) of *T. pratense* was strongly influenced by soil, AMF treatments and planting ratio (Table 2). In mixtures with *L. multiflorum*, the RYI_{ind} of *T. pratense* was depressed below its RY_{ind} in monoculture by 80 % in the high sand soil (Fig. 2A) and 90 % in low sand soil (Fig. 2B) in the absence of AMF. The presence of AMF significantly enhanced the RY_{ind} of *T. pratense* in both soils compared to the non-mycorrhizal treatment (all $p < 0.0001$, Fig. 2A and B), demonstrating that AMF reduced competitive pressure by *L. multiflorum*. In the high sand soil, both the high-diversity and *D. celata* AMF treatments were similar in effect ($p > 0.50$) and resulted in a RY_{ind} that did not differ from 1 ($p = 0.2$, Fig. 2A),

indicating that competitive effects of *L. multiflorum* depressing aboveground growth of *T. pratense* were completely alleviated in these two treatments. However, all other AMF treatments differed strongly from the high-diversity AMF treatment (all $p < 0.0001$). Intriguingly, the same effect was not seen in the low sand soil, where although all AMF treatments improved the RY_{ind} of *T. pratense*, all were significantly lower than 1 (all $p < 0.0001$, Fig. 2B). Furthermore, all *T. pratense* plants inoculated with single AMF had significantly lower RY_{ind} than the plants inoculated with the high-diversity treatment with all four AMF (all $p < 0.01$, Fig. 2B; Table 2).

The RY_{ind} of *L. multiflorum* was also found to be heavily influenced by soil conditions, AMF treatment and planting ratio (Table 2). In all cases, the RY_{ind} of *L. multiflorum* was significantly greater than 1 in both soils (all $p < 0.0001$) and the *L. multiflorum* individuals also obtained greater biomass when grown in mixture with *T. pratense* (Fig. 2C and D). Within the high sand soil, the RY_{ind} of *L. multiflorum* (Fig. 2C) showed an inverse ranking of AMF treatments in biomass production compared to that of *T. pratense* (Fig. 2A), demonstrating an AMF-mediated *T. pratense* competitive effect on *L. multiflorum*. Moreover, *L. multiflorum* RY_{ind} was significantly greater in the non-mycorrhizal treatment than all other AMF treatments (all $p < 0.05$, Fig. 2C). The RY_{ind} of *L. multiflorum* in the low sand soil was only significantly depressed below the non-mycorrhizal treatment in the presence of *D. celata* ($p < 0.01$). The effect of the high-diversity AMF treatment on the RY_{ind} of *L. multiflorum* also differed from that of *G. intraradices* ($p < 0.0001$, Fig. 2D).

The relative yield total (RYT) varied between soils as well as among AMF treatments and planting ratios (Table 2). Overyielding (RYT values > 1) occurred more frequently in the high sand soil resulting in an overall greater RYT than in the low sand soil (Fig 2E and F). Regardless of soil conditions, inoculation with *G. mosseae* and the non-mycorrhizal resulted in similar RYT values; both of which did not result in overyielding and differed significantly from inoculation with *G. intraradices* and the high-diversity AMF treatment (all $p \leq 0.01$, Fig. 2E).

TABLE 2. Results of the ANOVA testing for the effects of soil conditions (Soil), planting ratio (Ratio) and AMF treatment (AMF) on the relative yield per individual (RY_{ind}) of *L. multiflorum*, *T. pratense* and the relative yield total (RYT) in mixtures. Data were log-transformed prior to analyses to improve homoscedasticity in the data of all three measures. The greenhouse in which plants were grown is represented by the 'Block' effect

		T. pratense RY_{ind}		*L. multiflorum* RY_{ind}		RYT	
Source of variation	*df*	F	p	F	p	F	p
Block	1	2.61	0.11	5.16	0.02	5.08	0.03
Soil	1	609	< 0.0001	84.2	< 0.0001	10.1	0.002
Planting Ratio	2	15.8	< 0.0001	451	< 0.0001	4.61	0.01
AMF	5	171	< 0.0001	16.7	< 0.0001	8.80	< 0.0001
Soil x Ratio	2	7.98	0.0005	6.27	0.002	0.95	0.39
Soil x AMF	5	4.16	0.001	4.89	0.0003	1.63	0.15
Ratio x AMF	10	1.58	0.12	2.55	0.007	0.98	0.46
Soil x Ratio x AMF	10	1.09	0.37	1.00	0.44	0.89	0.55
Error	177						

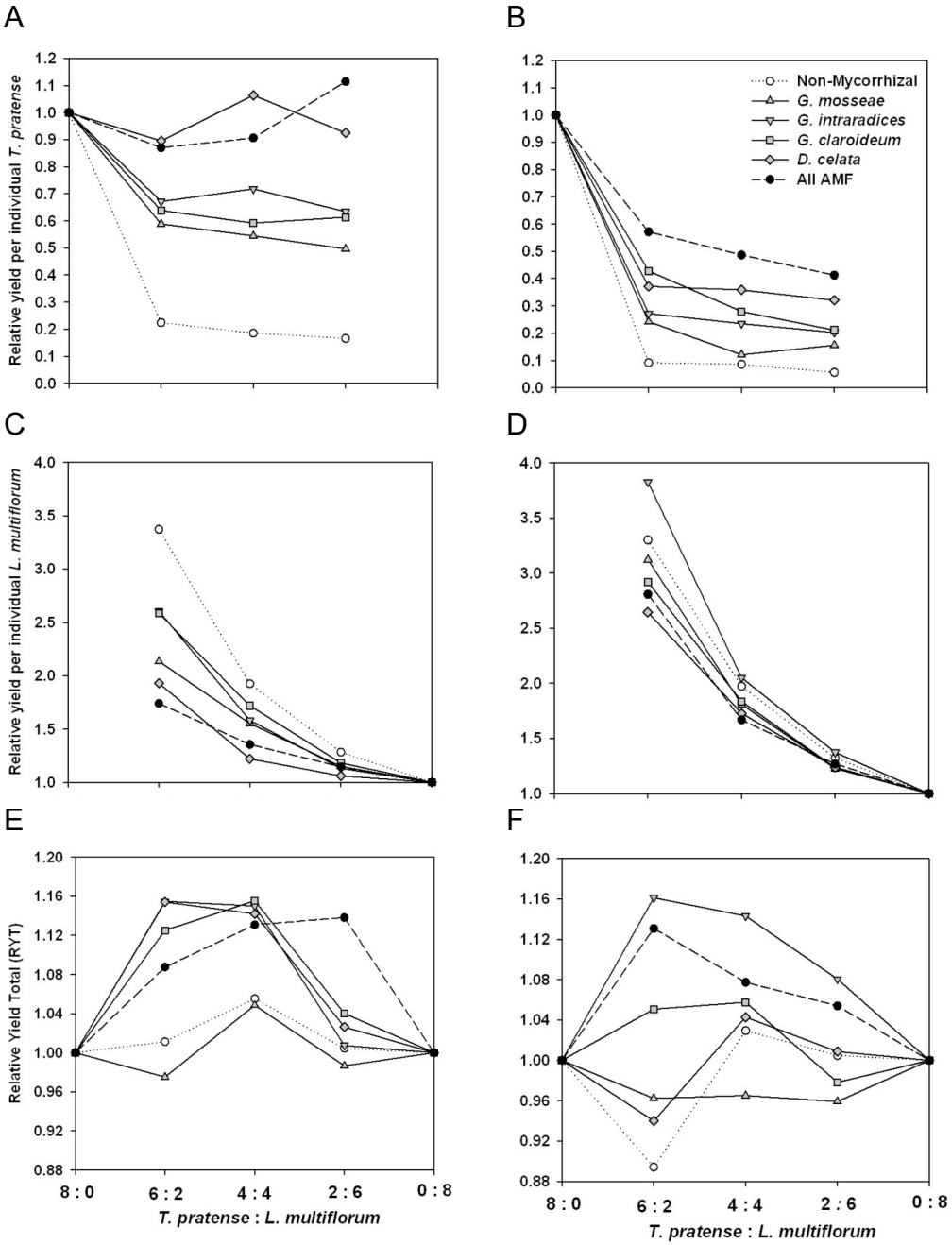

FIGURE 2. The relative yield per individual (RY_{ind}) of *T. pratense* (A and B) and *L. multiflorum* (C and D) as well as the relative yield total (RYT, E and F) in both high sand (A, C and E) and low sand (B, D and F) soils. All *T. pratense* RY_{ind} values differed from 1, with the exception of *D. celata* and All AMF treatments in the high sand soil (A). In all other cases, regardless of soil conditions, the RY_{ind} of *T. pratense* differed from 1. In all cases the RY_{ind} of *L. multiflorum* differed from 1. The RYT differed from 1 in all cases in the high sand soil (E) except for the non-mycorrhizal and *G. mosseae* treatments. Inoculation with *G. intraradices* and all four AMF resulted in RYT values significantly greater than 1 within the low sand soil (F).

Arbuscular mycorrhizal colonization

All AMF were found to colonize roots of host plants when inoculated individually. Irrespective of soil condition and planting ratio, *G. intraradices* colonized the greatest percentage of roots (79.5 %, SE = 1.5), followed by *G. claroideum* (35.1%, SE = 1.7), *D. celata* (22.0 % SE = 1.4) and *G. mosseae* (17.7 %, SE = 1.8). All four AMF were detected by qPCR in 43 of the 60 replicates where all four AMF were co-inoculated. In 7 cases *G. mosseae* and in 8 cases *G. claroideum* were not detected, while in two cases both *G. claroideum* and *G. mosseae* were not detected. *Glomus intraradices* was the most abundant within roots, when all four AMF were co-inoculated, with an average of 16.2×10^5 (SE = 1.84×10^5) LSU copies per mg of dried root, followed by *D. celata* (6.09×10^4, SE = 0.50×10^4), *G. claroideum* (5.66×10^4, SE = 1.07×10^4) and *G. mosseae* (2.87×10^3, SE = 0.72×10^3). The percent colonization and the number of LSU copies of each of the AMF taxa differed among planting ratios and soils depending on AMF taxa (see Appendix B). In general, all AMF taxa were abundant within the *T. pratense* monoculture and the high sand soil, with the exception of *G. claroideum*, which was more present within roots in the low sand soil and showed preference for the *L. multiflorum* monoculture in the high sand soil (Appendix B).

DISCUSSION

Our results demonstrate that AMF identity and diversity has a large impact on competitive interactions between the grass *L. multiflorum* and the legume *T. pratense,* favoring the legume. Moreover, in support of our hypothesis, the high-diversity AMF treatment with all four AMF in all but one case improved the biomass production of individual *T. pratense* plants more than did the individual AMF taxa, irrespective of soil conditions, enabling it to better coexist with *L. multiflorum* in mixtures (see Fig. 2).

The effect of AMF species identity and AMF diversity on plant productivity varied between the two soilss. For example, in the less productive high sand soil, the best single AMF species and the diverse AMF mixture had an equally beneficial effect on the competitive ability of the legume. Conversely, in the more productive low sand soil the diverse AMF species community was more beneficial than the best individual AMF in supporting legume competitive ability. The differences between soils in the relative importance of AMF species identity versus diversity provides support for the insurance effect of biodiversity (Yachi and Loreau 1999),

demonstrating the role of AMF richness to be an important mediator of plant productivity across heterogeneous soil conditions.

Effects of AMF on plant productivity between soils

Earlier work has shown that AMF and different AMF taxa alter competitive interactions and coexistence between plants (Fitter 1977, Hartnet et al. 1993, Hetrick et al. 1994, Zobel and Moora 1995, Scheublin et al. 2007, Schroeder-Moreno and Janos 2008, Collins and Foster 2009). However, effects of AMF diversity on competitive interactions were not yet investigated. This study now shows that AMF species richness indeed influences plant competition and that, especially in heterogeneous environments, the effects of a diverse AMF community may result in greater effects on the competitive interactions between plants than most of the individual AMF of which the AMF community is comprised.

Furthermore, our results show that AMF can enhance overall plant productivity by easing competitive interactions between plants resulting in overyielding. Within the more productive low sand soil the greater RY_{ind} of *T. pratense* in mixtures in the high-diversity treatment reveals a more diverse AMF community is of greater importance for plant co-existence than any individual AMF. This demonstrates complementarity within a diverse belowground community of mutualists to be a mechanism behind plant complementarity aboveground. However, the effect of the diverse AMF community on aboveground plant productivity within the high sand soil was similar to that of a single AMF taxa, *D. celata*, suggesting a sampling / selection effect within the high AMF diversity treatment may be behind the functioning of the AMF community (see, Wardle 1999 / Loreau and Hector 2001). This reveals AMF identity can be of greater importance than diversity *per se* depending on abiotic soil conditions.

Importantly, earlier studies investigating effects of AMF richness on plant coexistence and community structure (e.g. van der Heijden et al. 1998*a*, Klironomos 2000, Vogelsang et al. 2006) did not test whether the AMF taxa co-inoculated at the start of the experiment were still present at the end. Using quantitative PCR we could verify that in the majority of pots all four AMF inoculated at the start where still present at the end of the experiment. Moreover, the AMF taxa identified as being most effective (*D. celata*), was present in all co-inoculated pots at the end of the experiment. This provides for the first time empirical evidence in support of

conclusions that effects of a more diverse AMF community can be driven by the dominance of the single most effective AMF.

Competitive interactions between host plants

Our findings show *L. multiflorum* to have a strong interspecific competitive effect on *T. pratense* under both soil conditions tested, but particularly in the absence of AMF and in the more productive low sand soil. In the absence of AMF, the competitive suppression of growth in *T. pratense* by *L. multiflorum* was considerable. This corresponds to previous studies that observed *Lolium* spp. as a strong competitor (Stone et al. 1998, Hodge et al. 1999, Cralle et al. 2003). The depression in *L. multiflorum* productivity in AMF-inoculated treatments compared to the non-mycorrhizal treatment would suggest AMF supply soil resources toward *T. pratense* away from *L. multiflorum*. This result is similar to that of Fitter (1977), who found growth of *L. perenne* to be greatly reduced by AMF when competing belowground with *Holcus lanatus* resulting from AMF-mediated nutrient uptake. This effect of indirect competition has also been observed in other AMF-related plant competition studies where the species that was better able to utilize AMF associations to increase its own nutrient uptake caused a growth depression in the neighboring competing plant (Zobel and Moora 1995; Moora and Zobel 1996; Marler et al. 1999; Zabinski et al. 2002). This study also confirms previous studies that show strong positive responses by *Trifolium* species to AMF inoculation (Crush 1995, Li et al. 1997, Takacs et al. 2006, Wang et al. 2007, Sudová 2009) and that *T. pratense* depends heavily on AMF to acquire soil resources (e.g. up 53–65 % of soil P in the study by Feng et al. 2003).

The improved *L. multiflorum* growth in mixtures relative to monocultures is likely a response to reduced intraspecific competition as well as the fact that *T. pratense* can fix nitrogen, which subsequently may have increased *L. multiflorum* growth, in line with previous observations in grass–legume mixtures (Turkington and Klein 1991; Elgersma and Hassink 1997; Elgersma et al. 2000; Lucero et al. 1999) and a series of plant biodiversity experiments (Temperton et al. 2007, Wacker et al. 2009). However, due to inherent problems with the classic replacement series (see Connolly 1986; Snaydon 1991; Gibson et al. 1999; Jolliffe 2000 for a discussion) these two effects are inseparable in our study. In either case, the increased biomass production of individual plants of *L. multiflorum* and the alleviation of the *L. multiflorum* competitive suppression of *T. pratense* biomass production in the

presence of AMF resulted in overyielding in mixtures reflecting the relaxed plant-plant competition, where AMF benefits to *T. pratense* outweighed the negative effects on *L. multiflorum*.

Synthesis

Our results demonstrate that AMF taxa differ in their ability to influence interspecific plant competition and that a diverse community of AMF can ease plant competition to a greater extent than do the individual AMF taxa. This reveals that a diverse AMF community is a key driver of the productivity of grass–clover ecosystems by relaxing interspecific plant competition and contributing to overyielding (Hector et al. 2002). However, whether or not a diversity of AMF improved productivity more than the best single AMF was dependent on soil conditions. The differing relative effects of AMF diversity in the two soils points to the importance of AMF diversity as an insurance in heterogeneous soil environments, which to date has received surprisingly little attention. Moreover, the use of qPCR proved to be a useful tool in detecting and quantifying co-colonizing AMF and their combined roles in ecosystem functioning for future soil biodiversity studies. What remains clear is that there is still much to be uncovered regarding the role of AMF diversity within fluctuating and heterogeneous environments as is typical in many natural ecosystems.

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APPENDIX A: Molecular methods in detail

Roots not used for assessing AMF colonization microscopically were frozen in liquid nitrogen and lyophilized. A random sample of lyophilized root was selected (approximately 20-25 mg) and the weight of each sample recorded. Extraction of DNA was done using the Qiagen DNeasy plant mini kit following the manufacturer recommendations for the purification of total DNA from plant tissue (Qiagen Sciences, Germantown, Maryland, USA). The qPCR reactions were done using a LightCycler 2.0 (Roche Applied Science, Rotkreuz, Switzerland). Reagent amounts per reaction were: 4.55 µl H_2O, 0.18 µl forward primer (25 µM), 0.18 µl reverse primer (25 µM), 0.04 µl hydrolysis probe (25 µM), 1.8 µl Roche LightCycler® TaqMan® Master mix (Roche Applied Science, Rotkreuz, Switzerland) and 2.25 µl of template DNA. The sequences of primers and hydrolysis probes for the nuclear large ribosomal subunit (LSU) specific to *G. mosseae*, *G. intraradices* and *G. claroideum,* are outlined elsewhere (Thonar 2009). The oligonucleotides were synthesized at Microsynth (Balgach, Switzerland), hydrolysis probes were labelled with fluorescein and BHQ-1 quencher at the 5`and 3`s ends, respectively. The primers were purified by polyacrylamide gel electrophoresis and hydrolysis probes by preparative HPLC. For the qPCR quantification of LSU copies of *D. celata*, novel primers and a hydrolysis probe were designed. Forward primer sequence was 5`-TCGGAGGTTGTAAAATACTTGG-3`, the reverse primer was 5`-CAAAGGCATTTGCTGCAATC-3`, hydrolysis probe sequence was 5`-AAGGTCTATAACACTCTCCCGAAG-3`. This combination targeted a 106-bp fragment of LSU of *D. celata*. Specificity of amplification was confirmed using genomic DNA preparation from spores of 19 different AMF strains as well as root colonized by the different AMF species included in this study (data not shown). The PCR program for *G. intraradices* and *G. claroideum* consisted of an initial denaturation and Taq-polymerase activation step at 95 °C for 15 min, followed by 50 cycles of: denaturation at 95 °C for 15 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 1 s. Following the 50 cycles, the program concluded by cooling at 40 °C for 30 s. This same program structure was used for *G. mosseae* and *D. celata* with an annealing conditions of 54 °C for 10 s and 62 °C for 15 s, respectively.

The cycle threshold (*Cp*) values as determined by the automatic function using the LighCycler software were recorded and used to estimate the number of LSU copies per mg of lyophilized root by the equation.

$$LSU = \frac{200 \times 10\text{^}\left[(b - Cp)/a\right]}{M_R}$$

Where M_R is the mass of lyophilized root in mg used for DNA extraction and *a* and *b* are constants derived from the calibration equations of each AMF primer and hydrolysis probe set. The calibration of each primer set used was done using serial dilution in series of 10 × dilutions of a plasmid carrying target DNA sequence, with a known concentration (see Jansa et al. 2008 for determining DNA concentration) and plotting the regression line between the log of the concentration of template DNA and the Cp of the reaction curve. The slope of the regression line is the constant *a* and the intercept is the constant *b* used in equation 1. The factor 200 stands for DNA preparation volume (in microliters), the calibration was derived as number of LSU copies per microliter template. In samples where inoculated AMF were not detected the number of LSU copies per mg of lyophilized root was assumed to be zero and was analyzed as such within all statistical calculations.

APPENDIX B: Mean root colonization levels and LSU copy number

Mean percent root length colonization (%) of each AMF when inoculated alone (single AMF taxa treatments) and the mean number of LSU copies (LSU) detected by qPCR of each AMF when co-inoculated (the treatment inoculated with all four AMF taxa). The standard error of the mean is shown in parentheses. For the comparison among planting ratios means not sharing the same letter are considered to differ significantly (Tukey HSD, $p < 0.05$). Similarly, a significant difference in the overall mean between soils is indicated by astricts (*)

High sand		*G. claroideum*		*G. intraradices*		*G. mosseae*		*D. celata*	
T. pratense	*L. multiflorum*	%	LSU $\times10^4$	%	LSU $\times10^5$	%	LSU $\times10^3$	%	LSU $\times10^4$
8	0	20.0^{a}	0.60	89.6^{a}	36.8	34.7^{a}	4.68	25.5	5.11
		(1.7)	(0.4)	(2.1)	(11.4)	(4.1)	(3.5)	(3.5)	(0.8)
6	2	25.2^{abc}	1.53	85.4^{ab}	14.7	22.5^{ab}	1.34	19.7	7.71
		(2.5)	(0.7)	(2.6)	(3.6)	(3.6)	(0.9)	(2.5)	(2.7)
4	4	24.0^{ab}	0.37	84.1^{ab}	10.6	13.3^{b}	2.70	16.7	9.77
		(3.4)	(0.2)	(3.6)	(2.8)	(3.8)	(1.0)	(4.1)	(1.5)
2	6	31.6^{bc}	0.81	82.2^{ab}	11.7	16.2^{b}	2.12	20.7	7.19
		(3.2)	(0.3)	(2.3)	(3.0)	(3.2)	(0.8)	(3.0)	(2.0)
0	8	35.8^{c}	4.73	75.1^{b}	19.4	20.6^{ab}	11.1	21.2	6.68
		(2.5)	(2.3)	(3.1)	(6.8)	(4.2)	(4.8)	(4.0)	(0.8)
	Overall mean	27.3*	1.61*	83.5*	18.6	21.5*	4.39*	20.8	7.29*
		(1.6)	(0.5)	(1.4)	(3.2)	(2.1)	(1.3)	(1.5)	(0.8)

Low sand		*G. claroideum*		*G. intraradices*		*G. mosseae*		*D. celata*	
T. pratense	*L. multiflorum*	%	LSU $\times10^4$	%	LSU $\times10^5$	%	LSU $\times10^3$	%	LSU $\times10^4$
8	0	48.5	21.0^{a}	97.4^{a}	25.2	42.8^{a}	2.51	36.7^{a}	8.48
		(5.8)	(5.10)	(1.0)	(4.2)	(2.7)	(1.5)	(6.0)	(1.4)
6	2	47.4	3.92^{c}	73.4^{b}	9.10	7.8^{b}	0.48	18.3^{ab}	3.61
		(6.9)	(0.84)	(3.3)	(3.1)	(2.7)	(0.4)	(1.7)	(1.1)
4	4	43.3	14.9^{ab}	72.3^{b}	15.3	10.3^{b}	3.04	24.9^{ab}	4.59
		(2.3)	(3.39)	(2.4)	(2.9)	(2.7)	(1.5)	(5.0)	(0.8)
2	6	33.4	6.59^{bc}	63.7^{b}	9.80	5.5^{b}	0.32	23.1^{ab}	3.85
		(6.3)	(1.68)	(5.2)	(2.5)	(1.0)	(0.2)	(6.3)	(0.8)
0	8	41.5	2.11^{c}	71.2^{b}	9.76	3.7^{b}	0.35	15.8^{b}	3.93
		(3.7)	(0.67)	(3.9)	(3.6)	(1.3)	(0.2)	(2.0)	(1.5)
	Overall mean	42.8*	9.72*	75.6*	13.8	14.0*	1.34*	23.3	4.89*
		(2.4)	(1.8)	(2.6)	(1.8)	(2.9)	(0.5)	(2.3)	(0.6)

Chapter 2

BELOWGROUND BIODIVERSITY EFFECTS OF PLANT SYMBIONTS SUPPORTS ABOVEGROUND PRODUCTIVITY

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ABSTRACT

Soil microbes play key roles in ecosystems, yet the impact of their diversity on plant communities is still poorly understood. Here we demonstrate that the diversity of belowground plant-associated soil fungi promotes plant productivity and plant coexistence. Using additive partitioning of biodiversity effects developed in plant biodiversity studies, we demonstrate that this positive relationship can be driven by complementarity effects among soil fungi in one soil type, and by a selection effect resulting from the fungal species that stimulated plant productivity the most in another soil type. Selection and complementarity effects among fungal species contributed to improving plant productivity up to 82 % and 85 %, respectively, above the average of the respective fungal species monocultures depending on the soil in which they were grown. These results also indicate that belowground diversity may act as insurance for maintaining plant productivity under differing environmental conditions.

INTRODUCTION

Recent work has highlighted the role of biological diversity as a regulator of ecosystem functions (Zavaleta *et al*. 2010). In grassland ecosystems, greater plant species richness is often associated with increased plant productivity (Hector *et al.* 1999; Tilman *et al*. 2001). This positive effect of biodiversity can be explained by the presence of a particular productive species driving a species-rich community (selection effect), as well as niche differentiation and facilitative interactions (complementarity effect) in more species-rich communities (Hooper *et al.* 2005). Both these effects can operate simultaneously and sum to the net effect of biodiversity on ecosystem functioning (Loreau & Hector 2001). The majority of such studies focusing on biodiversity–ecosystem functioning have focused on aboveground organisms while relatively few address the role of biodiversity in the belowground soil microbial community (Balvanera *et al.* 2006). Furthermore, the use of additive partitioning of biodiversity effects has yet to be used to assess the functioning of soil microbial communities.

Soil microbes play critical roles in a number of ecosystem processes and services and several reports emphasize the major role soil microbial diversity plays in sustaining ecosystem functioning (Wardle *et al.* 2006; van der Heijden *et al*. 2008). Deciphering the importance of soil microbial diversity is a key issue in ecology as many studies have found belowground diversity to be reduced by anthropogenic effects, particularly agricultural practices (Helgason *et al*. 1998; Jansa *et al.* 2002; Van der Wal *et al.* 2006). For instance, a number of studies have shown that richness of arbuscular mycorrhizal (AM) fungi is reduced by agricultural intensification (Oehl *et al*. 2004; Verbruggen *et al*. 2010). These fungi are a group of obligate root endophytic fungi ubiquitous in most terrestrial ecosystems (Smith & Read 2008).

Previous studies have shown that plant productivity and diversity are functions of increasing AM fungal richness (van der Heijden *et al.* 1998; Maherali & Klironomos 2007). This generally is attributed to complementarity among AM fungi that is explained by niche segregation and facilitation. There is evidence that complementarity in their function may occur as different phylogenetic groups of AM fungi possess different life strategies and have different host effects (Maherali & Klironomos 2007). Moreover, a high degree of genetic and functional variability can occur within an AM fungal community (Koch *et al*. 2006). Such multifunctionality of AM fungi could translate to greater host benefits in more AM fungi-rich communities

as each could theoretically provide additional services to host plants that contribute to greater plant productivity (Koide 2000).

Conversely, the single most productive AM fungal species can have a similar effect as mixtures of AM fungi (Vogelsang *et al.* 2006). This suggests that the relationships between AM fungal richness and plant productivity may be driven by the selection effect, whereby the likelihood of including the fungal species with the strongest effect on functioning increases with AM fungal richness; also referred to as a 'sampling effect' (see Huston 1997). However, it is impossible to assess either selection or complementarity effects (*sensu* Loreau & Hector 2001) of AM fungal richness without knowing which fungi contributed to aboveground effects in mixed communities of AM fungi. To date no study has partitioned out the complementarity and selection effects in AM fungal communities and as a result, it is unclear how these effects influence AM fungal richness–plant productivity relationships. This has generated some debate regarding the mechanisms behind AM fungal richness–plant productivity relationships (e.g. Wardle 1999; van der Heijden *et al.* 1999).

There are a number of additional spatial, temporal and environmental factors that can influence the functioning of AM fungal communities within soils. For example, soil abiotic conditions are known to shift AM fungal community assembly (Lekberg *et al.* 2007) and the mycorrhizal relationship (Johnson *et al.* 1997). This is likely an additional factor resulting in the observed variation of the effects of AM fungal diversity on plant hosts. For instance, soil resource availability may determine whether soil fungi can coexist (Kennedy 2010) and may thus influence whether the effect of AM fungal diversity is driven by a complementarity or selection effect. The relationship between AM fungi and plant communities under differing environmental characteristics is also crucial with regard to the question whether species richness of AM fungi may act as insurance for stability in ecosystem functioning in spatially heterogeneous environments or under altered environmental conditions (Yachi & Loreau 1999).

In the present study we use a grass-clover model system to test whether AM fungal richness enhances plant productivity in two different soils to reveal the impact that the abiotic soil environment can have on AM fungal richness–plant productivity relationships. Subsequently, we assess whether the AM fungal richness–plant productivity relationships are due to a complementarity or selection effect using additive partitioning (Loreau & Hector 2001). Since, the identification of each species present and its effect on the ecosystem response in both monocultures and mixtures

is a pre-condition for partitioning selection and complementarity effects (Loreau & Hector 2001), we used quantitative PCR to detect the abundance of the AM fungi in monocultures and in mixtures in which four AM fungal taxa were inoculated in all possible combinations. These data enabled us to estimate the relative contribution to aboveground plant productivity of each AM fungal species in mixtures such that additive partitioning of biodiversity effects could be used to assess the role of selection and complementarity effects in increasingly rich mixtures of AM fungi.

METHODS

Preparation of fungal inocula, plants and soils

We used four AM fungi, *Glomus intraradices* (isolate BEG 21, see van der Heijden *et al.* 2006 for description), *G. mosseae* (isolate BEG161, Jansa *et al.* 2002), *G. claroideum* (isolate JJ132, Jansa *et al.* 2002) and *Diversispora celata* (FACE 234, Gamper *et al.* 2009); each belonging to a unique *Glomus* group; Aa, Ab, B and C, respectively (Schüssler *et al.* 2001, Gamper *et al.* 2009). These fungi occur in Swiss agricultural and grassland ecosystems and can differ in function (Jansa *et al.* 2005; Thonar *et al*. 2010). Inocula, including the non-mycorrhizal control inoculum, were propagated on *Plantago lanceolata* L. for 6 months after which the soil was dried and roots were cut into < 5 cm pieces and homogenously mixed. *Glomus claroideum, G. intraradices*, *G. mosseae* and *D. celata* inocula were observed colonizing 30.4 %, 90.5 %, 42.1 % and 17.5 % of the root length of *P. lanceolata* with 22, 38, 2 and 121 spores per cm^3 of soil, respectively. No AM fungal propagules were observed in the control inoculum.

The field soil used for the experiment was collected from a natural grass-clover field located at Agroscope Reckenholz research station in Zürich, Switzerland (047° 25' 38.71'' N, 8° 31' 3.91'' E), sieved through a 0.5 cm mesh and mixed with quartz sand in the ratios of 1:4 and 4:1 soil to sand (v/v) to create the two soil types, "high-sand" and "low-sand" respectively. Soils were sterilized by autoclaving for 99 min at 121 °C. The soil characteristics are provided in Appendix A.

Pots, 1 L in volume, were filled with 1.15 kg (dry weight) of one of the two soil types. Pots were inoculated with AM fungi in all possible combinations including a non-mycorrhizal control producing AM fungal richness treatments from 0 to 4 for a total of 16 AM fungal treatments. All pots received 50 ml of inoculum, thus, pots inoculated with 2, 3 or 4 AM fungi received approximately 25 ml, 16.5 ml and 12.5 ml of inoculum of each fungal species respectively. Soil and inoculum were mixed

thoroughly in each pot to create a homogenous substrate and each soil × AM fungal treatment was replicated six times resulting in 192 pots. Pots were randomly distributed in two adjacent greenhouses.

We used *Trifolium pratense* L. var. Milvus and *Lolium multiflorum* Lam. var. Daxus as the host plant community. We chose this grass-legume community for its key role in both agricultural and natural grassland ecosystems where the species of the two involved plant functional groups commonly coexist (e.g. Nyfeler *et al*. 2009). Seeds, originating from agricultural plots located at Agroscope Reckenholz research station, Zürich, Switzerland, were surface sterilized by agitation in 1.25 % sodium hypochlorite for 5 minutes, followed by rinsing in H_2O and placed on 1.5 % water agar until germination. Four seedlings of each plant species were planted into each pot. Seedlings not surviving transplant were replaced within 2 weeks post planting. In order to standardize the non-mycorrhizal microbial community with a natural soil microbe community native to a natural grass-clover-field, a microbial wash was created by sieving 1 L fresh field soil with 5 L H_2O through a series to < 11 μm of which 10 ml was added to each pot. Numerous root nodules were observed on clover indicating this microbial wash contained active micro-organisms including nodule-inducing rhizobia.

Plants were grown for 25 weeks with 16 h / 25 °C days under natural light maintained above 300 W/m^2 by 400-W high pressure sodium lights, and 8 h / 16 °C nights and received H_2O to maintain soil moisture at 10–20 % by weight.

Data collection

After 9 and 16 weeks shoots were cut 4 cm above the soil surface to simulate mowing. The final harvest occurred at 25 weeks and plants were cut directly at the soil surface. Plant material from each harvest was separated into species, dried at 70 °C and weighed. Data were pooled across harvests for all subsequent analyses, as biomass responses were similar at all harvests (data not shown). At the final harvest, roots were washed and stored at –20 °C until they could be processed for microscopy and DNA extraction. Frozen roots were thawed and cut into small fragments (1–2 cm). For microscopy, a random subsample of 1–2 g of fresh roots was cleared and stained with 5 % pen-ink vinegar as described in Vierheilig *et al.* (1998). Stained roots were scored for the presence of colonization by AM fungi using the intersect method outlined in McGonigle *et al.* (1990) for 100 intersects.

For DNA extraction, roughly half the remaining root fragments were randomly selected, containing a representative root sample of both host plants, and lyophilized. Approximately 20–25 mg of lyophilized roots were used for DNA extraction using the Qiagen DNeasy plant mini kit following manufacturer recommendations for the purification of total DNA from plant tissue (Qiagen Sciences, Germantown, Maryland, USA). The primer sequences and hydrolysis probes used were specific to each of the four AM fungi and targeted the nuclear large ribosomal subunit (LSU). The qPCR reactions were carried out using the LightCycler 2.0 (Roche Applied Science, Rotkreuz, Switzerland) and the cycle threshold values were used to determine the number of LSU copies per mg of lyophilized root (see Appendix A). The primers and probes used did not show any interference from the presence of non-target AM fungal DNA (Appendix B). Further details on the primers and probes used, as well as the reagents and cycling conditions for the qPCR are described elsewhere (Thonar 2009; Wagg *et al.* 2011a).

Contribution of AM fungi to plant productivity

We estimated the contribution of each AM fungus to aboveground plant biomass in the AM fungal mixtures by weighting each of the AM fungal species in the mixture according to its relative abundance (*RY*) and the aboveground biomass the plants produced when mono-inoculated (β). The *RY* of each AM fungal species in the mixture was calculated as the observed abundance of an AM fungus in the mixture (*O*) proportional to its average abundance in monoculture (*M*); equivalent to the relative yield outlined by de Wit (1960), such that $RY = O/M$. The *RY* of each AM fungus was then multiplied by β (the aboveground biomass in the respective mono-inoculated plants). The contribution to aboveground plant biomass (*P*) of each AM fungus in the mixture was then calculated as

$$P_i = \frac{RY_i \cdot \beta_i}{\sum_{i=1-n} RY_i \cdot \beta_i} \times \alpha$$

where RY_i is the relative abundance of AM fungal species *i* in the mixture, β_i is the aboveground plant biomass when mono-inoculated with this fungus *i* and α is the aboveground plant biomass produced in the AM fungal mixture. By using this weighting method we assume that the relative abundance of an AM fungus in the mixture also reflects its influence on plant biomass in the mixture relative to its effect in monoculture regardless of other factors, such as enhanced or reduced abundance

of other fungi (e.g. symbiotic efficiency per unit biomass is not dependent on the abundance of other AM fungi).

Biodiversity effects

For each treatment with two or more AM fungi, additive partitioning (Loreau & Hector 2001) was used to determine the biodiversity effects of AM fungi colonizing the belowground root system on the aboveground biomass of *T. pratense*, *L. multiflorum* and their combined total. The net biodiversity of AM fungi on plant productivity was defined as the difference between the plant biomass observed in AM fungal mixtures and the average plant biomass observed in AM fungal monocultures of the fungal species making up the mixture. The net biodiversity effect was then partitioned into a selection effect (strong influence of particular AM fungal species in mixture) and a complementarity effect (several AM fungal species contributing more to plant productivity in AM fungal mixtures than expected from their AM fungal monocultures) following Loreau & Hector (2001).

The estimated contribution to plant biomass (*P*) was used as the 'observed yield' for each AM fungal species in the mixture. The plant biomass in the mono-inoculated treatments was used as the 'monoculture yield'. The 'expected yield' of each AM fungi was calculated as plant biomass in the mono-inoculated treatment divided by the number of AM fungi in the mixture, such that the sum of the 'expected yield' of the AM fungi in the mixed inoculum treatment was equal to the average of the biomass produced in mono-inoculated treatments following the null hypothesis of Loreau & Hector (2001). It should be noted that an increased effect of an AM fungal mixture over the best mono-inoculated AM fungus, analogous to 'transgressive overyielding' in plant biodiversity studies, is only one possible outcome of complementarity (see Hector *et al*. 2002).

Data Analyses

The effect of soil × AM fungal treatment on the aboveground biomass of *T. pratense*, *L. multiflorum* as well as their combined total was assessed by ANOVA with soil type, AM fungal treatment, initial AM fungal richness, realized AM fungal richness, and the interactions with soil type as sources of variation. The non-mycorrhizal treatment was removed from the data in the analysis in order to remove any confounding influence it may have in assessing AM fungal diversity. Both initial and realized AM fungal richness were used in the model since not all AM fungi could

be detected after harvest by qPCR in 18 of the 180 pots inoculated with multiple AM fungi (see Appendix D for details). The variation among AM fungal treatments and their interaction with soil, if significant, was then partitioned out by contrasts using step-wise addition of contrast terms (presence/absence of a fungus in all combinations), in order by which they explained the greatest amount of variation with fewest interactions. The abundance of AM fungi, their interactions with each other and soil treatment were then added to the model. Step-wise deletion of terms was used to test for linear and non-linear relationships after accounting for the presence/absence of an AM fungus. Contrasts within separate ANOVAs for each soil treatment were used to test differences between mycorhizal and non-mycorrhizal treatments as well as whether AM fungal mixtures differed from the corresponding AM fungal monoculture that had the strongest effect on plant productivity in order to detect any potential facilitative effects.

The abundance of each AM fungal species was assessed using a two-way ANOVA with soil and AM treatments as well as their interaction as sources of variation. In order to determine whether AM fungi were significantly altered in abundance in AM fungal mixtures, the relative abundance (*RY*) of each AM fungus was assessed for each soil separately by one-way ANOVA with AM fungal treatment as the source of variation. The *RY* was log transformed to improve homoscedasticity in the data that produced positive and negative values such that a difference from 0 (no change in abundance) could conveniently be tested.

All three biodiversity effects (net, selection and complementarity) were assessed by ANOVA using soil type, AM fungal treatment and AM fungal richness (both initial and realized) as well as the interaction of soil type with AM fungal treatment and richness as sources of variation. Separate regression models for each biodiversity effect in each soil type were then used to determine whether the magnitude of effects corresponds with increasing AM fungal richness. The greenhouse in which plants were grown was added as a block effect in all ANOVA models. All statistics were calculated using R 2.11.1 (The R Foundation for Statistical Computing 2010).

RESULTS

Responses in aboveground plant productivity

Overall, AM fungal richness enhanced aboveground biomass of grass-clover mixtures (Fig. 1a) resulting from a positive effect of AM fungal richness on *T. pratense* biomass (Fig. 1b) and a marginal negative effect on *L. multiflorum* biomass (Fig. 1c). The realized AM fungal richness explained a greater proportion of variation in all three measures of aboveground biomass than the initial AM fungal richness, which did not explain any further variation in biomass after the realized richness was entered into the model. Thus, initial AM fungal richness is not present in the model (Table 1). Overall, the presence of AM fungi altered the relative abundance of *T. pratense* biomass from 3 % and 11 % in the non-mycorrhizal treatment up to 27 % and 66 % in the most beneficial AM fungal treatment in the low- and high-sand soils, respectively.

Soil and AM fungal treatment strongly influenced all three biomass measures (Table 1). Overall, *T. pratense* produced greater biomass in the high-sand soil and *L. multiflorum* produced greater biomass in the low-sand soil (Fig. 1). Presence of AM fungi significantly enhanced *T. pratense* biomass in both soils (high-sand: $F_{1,79} = 268$, $p < 0.001$, low-sand: $F_{1,79} = 85.4$, $p < 0.001$, Fig. 2). The presence and abundance of *D. celata* significantly influenced *T. pratense* biomass resulting in a 9–10 fold increase above non-mycorrhizal treatments in monoculture in both soils (Table 1a; Fig. 2). Moreover, the effect of *D. celata* presence on *T. pratense* biomass depended on soil and AM fungal treatment (Table 1a; Fig. 2). Combinations of AM fungi, particularly those involving *G. claroideum* and *G. intraradices*, resulted in greater effects on *T. pratense* biomass than the most effective AM fungi in mono-inoculated plants (Fig. 2). *Lolium multiflorum* biomass was reduced in the presence of AM fungi by approximately 20–40 % and 15–20 % in the high- and low-sand soils respectively (Fig. 2) compared to the non-mycorrhizal treatment (high-sand: $F_{1,79} = 28.5$, $p < 0.001$, low-sand: $F_{1,79} = 13.9$, $p < 0.001$). The presence of *G. claroideum* in AM fungal treatments contributed to the largest depressions in *L. multiflorum* growth followed by *G. intraradices* (Table 1b, Fig. 2). Additionally, the abundance of *G. mosseae* significantly influenced *L. multiflorum* biomass (Table 1b). The effects of the various AM fungal combinations as well as the relationships in abundance are also evident in the analysis of the combined biomass of the two plant species (Table 1c).

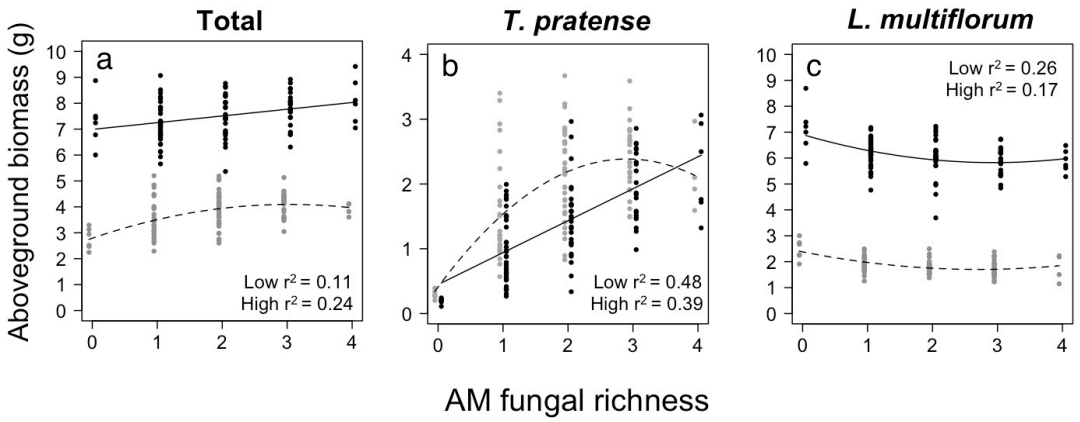

Figure 1. Scatter plots with trend lines showing the relationship of the aboveground biomass of a) Total aboveground biomass, b) *T. pratense* and c) *L. multiflorum* with realized AM fungal richness in both low-sand (black dots, solid line) and high-sand (grey dots, dashed line) soils. All models were significant (all $p < 0.001$).

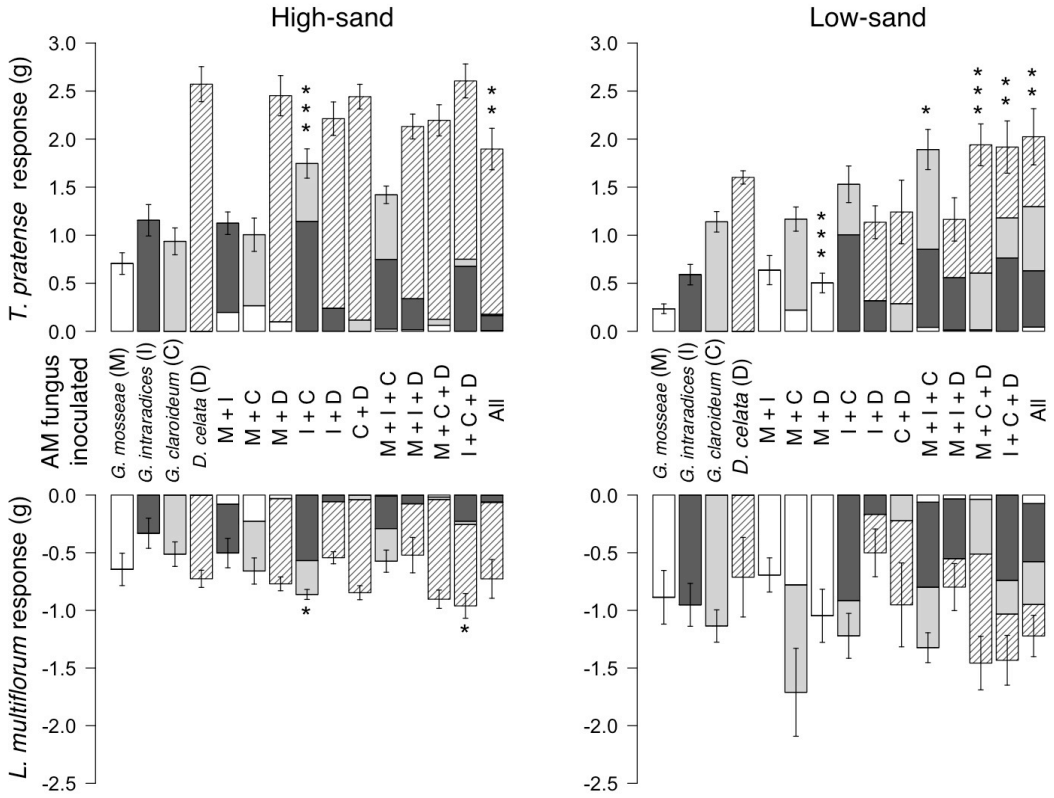

Figure 2. The mean and standard error for the difference between mycorrhizal and non-mycorrhizal biomass of *T. pratense* (above) and *L. multiflorum* (below) in both soils (high-sand non-mycorrhizal *T. pratense* = 0.26 g, SE = 0.03 g and *L. multiflorum* = 2.47 g, SE = 0.16 g; low-sand non-mycorrhizal *T. pratense* = 0.19 g, SE = 0.02 g and *L. multiflorum* = 7.11 g, SE = 0.39 g). The estimated contribution of each AM fungus to the effect of AM fungal inoculation on aboveground plant biomass is represented by the proportional shading of each bar, reflecting their relative abundance in the mixture as well as their influence on growth in relation to their monocultures. The presence of an AM fungus is indicated on the x-axis (C = *G. claroideum*, I = *G. intraradices*, M = *G. mosseae*, D = *D. celata*). Differences (either greater or less) in plant biomass between a mixed-inocula treatments and the corresponding most effective mono-inoculated AM fungus is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 1. Summary of ANOVA results for the biomass of a) *T. pratense*, b) *L. multiflorum* and c) their combined total. The significant proportion of the variance explained by the different AM fungal treatments (using only inoculated treatments) and the interaction with soil type is partitioned out by the addition of contrast terms explaining the greatest amount of variation with the fewest terms (indented terms). Transformations were used to improve homoscedasticity in the data (*T. pratense* square-root transformation and *L. multiflorum* transformed to the power of 0.25). Capital letters in contrasts represent the presence or abundance of an AM fungus (M = *G. mosseae*, I = *G. intraradices*, C = *G. claroideum* and D = *D. celata*). Significance levels of the *F*-test are indicated as: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

a) *T. pratense* biomass

Source of Variation	d.f.	MS	*F*
Block	1	0.32	12.2 ***
Soil	1	2.89	109 ***
Realized richness	1	3.92	148 ***
AM fungal treatments	**14**		
D presence	1	1.62	61.0 ***
M presence	1	1.19	44.9 ***
I presence	1	0.16	5.89 *
I × D presence	1	0.56	21.0 ***
C × D presence	1	0.34	12.7 ***
I × C × D presence	1	0.18	6.61 *
AM residual	8	0.05	1.83
D abundance	1	0.24	9.18 **
Soil × AM	**14**		
Soil × D presence	1	0.54	20.3 ***
Soil × C presence	1	0.68	25.5 ***
Soil × M × C presence	1	0.25	9.53 **
Soil × AM residual	11	0.04	1.34
Residuals	146	0.03	

b) *L. multiflorum* biomass

Source of Variation	d.f.	MS	F
Block	1	6.84×10^{-5}	0.04
Soil	1	7.57	4.62×10^{3} ***
Realized richness	1	1.82×10^{-2}	11.1 **
AM fungal treatments	**14**		
C presence	1	2.52×10^{-2}	15.4 ***
I presence	1	0.81×10^{-2}	4.69 *
AM residual	12	0.21×10^{-2}	1.26
M abundance	1	0.91×10^{-2}	5.58 *
Soil × AM	**14**	0.23×10^{-2}	1.38
Residuals	146	0.16×10^{-2}	

c) Total biomass

Source of Variation	d.f.	MS	*F*
Block	1	1.31×10^{-2}	10.8 **
Soil	1	2.88	2.38×10^{3} ***
Realized richness	1	5.64×10^{-2}	46.7 ***
AM fungal treatments	**14**		
D presence	1	11.3×10^{-2}	93.8 ***
M presence	1	2.67×10^{-2}	22.1 ***
C presence	1	0.78×10^{-2}	6.45 *
I × D presence	1	1.35×10^{-2}	11.2 **
M × I presence	1	1.17×10^{-2}	9.70 **
C × D presence	1	0.76×10^{-2}	6.29 *
AM residual	8	0.06×10^{-2}	0.50
D abundance	1	0.93×10^{-2}	7.72 **
M abundance	1	1.17×10^{-2}	9.69 **
Soil × AM	**14**		
Soil × D presence	1	3.25×10^{-2}	26.9 ***
Soil × AM residual	13	0.16×10^{-2}	1.29
Soil × M abundance	1	0.64×10^{-2}	5.29 *
Soil × M non-linear abundance	2	0.58×10^{-2}	4.78 **
Residuals	142	0.12×10^{-2}	

AM fungal abundance in roots

All four AM fungi colonized roots when mono-inoculated, demonstrating their viability. Of the four AM fungi used, *G. intraradices* was the most infective, although not the most beneficial, in monoculture, colonizing 79.5 % (SE = 1.54) of root length, pooled for both soils, followed by *G. claroideum*, *D. celata* and *G. mosseae* which colonized 35.1 % (SE = 1.74) %, 22.0 % (SE = 1.37) and 17.7 % (SE = 1.82) of root length, respectively. No AM fungal colonization was observed in any of the roots of control plants. The root-length colonization by AM fungi correlated well with the number of LSU copies detected (Spearman's rho = 0.74, $p < 0.001$, pooled for all mono-inoculated treatments). The abundance of each AM fungal species differed between soils and among AM fungal treatments (Appendix C). Generally, *G. intraradices* was most affected by other AM fungi while the abundances of *G. mosseae*, *G. claroideum* and *D. celata* were most affected by the soil in which they were inoculated (Appendix C). Overall, the abundance of each AM fungus was frequently less in AM fungal mixtures than their respective mono-inoculated treatments (Appendix D).

The abundance of *G. claroideum* and *G. mosseae* was significantly less than its abundance in monoculture in all AM fungal mixtures in both soils (Appendix E). Intriguingly, *D. celata* did not deviate significantly from its abundance in monoculture

in the majority of AM fungal mixtures in the high-sand soil (Appendix E), but was significantly reduced in abundance in all AM fungal treatments, including complete absence in the presence of *G. mossseae*, in the low-sand soil (Appendix E). *Glomus intraradices* was also absent in roots of all replicates when co-inoculated with *G. mosseae*, but in all other AM fungal mixtures did not differ significantly from its inoculation in monoculture in the low-sand soil (Appendix E).

Biodiversity effects

Analysis of the biodiversity effects of AM fungi on aboveground plant productivity revealed soil and AM fungal combination to be important factors influencing the complementarity and selection effects, particularly in the case of *T. pratense* (Appendix F). For example, combinations with *G. claroideum* and *G. intraradices* resulted in complementarity, as evidenced by their mixtures having a greater effect than the average of their mono-inoculated treatments (Fig. 2). Combinations involving *D. celata* always resulted in a strong selection effect in the high-sand soil (Fig. 2).

The selection effect in AM fungal mixtures on all three measures of aboveground plant productivity differed between the two soils (Appendix F). In the high-sand soil the selection effect accounted for up to 82 % of the net biodiversity effect on *T. pratense.* The selection effect had an overall significant positive effect on *T. pratense* and total plant biomass while an overall negative selection effect on *L. multiflorum* biomass (Fig. 3). The grand mean of the selection effect in the low-sand soil did not differ from 0 (Fig. 3). The complementarity effect on *T. pratense* accounted for up to 85 % of the net biodiversity effect in the low-sand soil and increased with AM fungal richness (Fig. 3) demonstrating increases in AM fungal richness correspond to increases in a complementarity effect. Overall, the effect of AM fungal richness resulted in a positive net effect on the biomass of *T. pratense* that outweighed the overall negative effects on *L. multiflorum* resulting in a significant positive effect on the total aboveground biomass (Fig. 3).

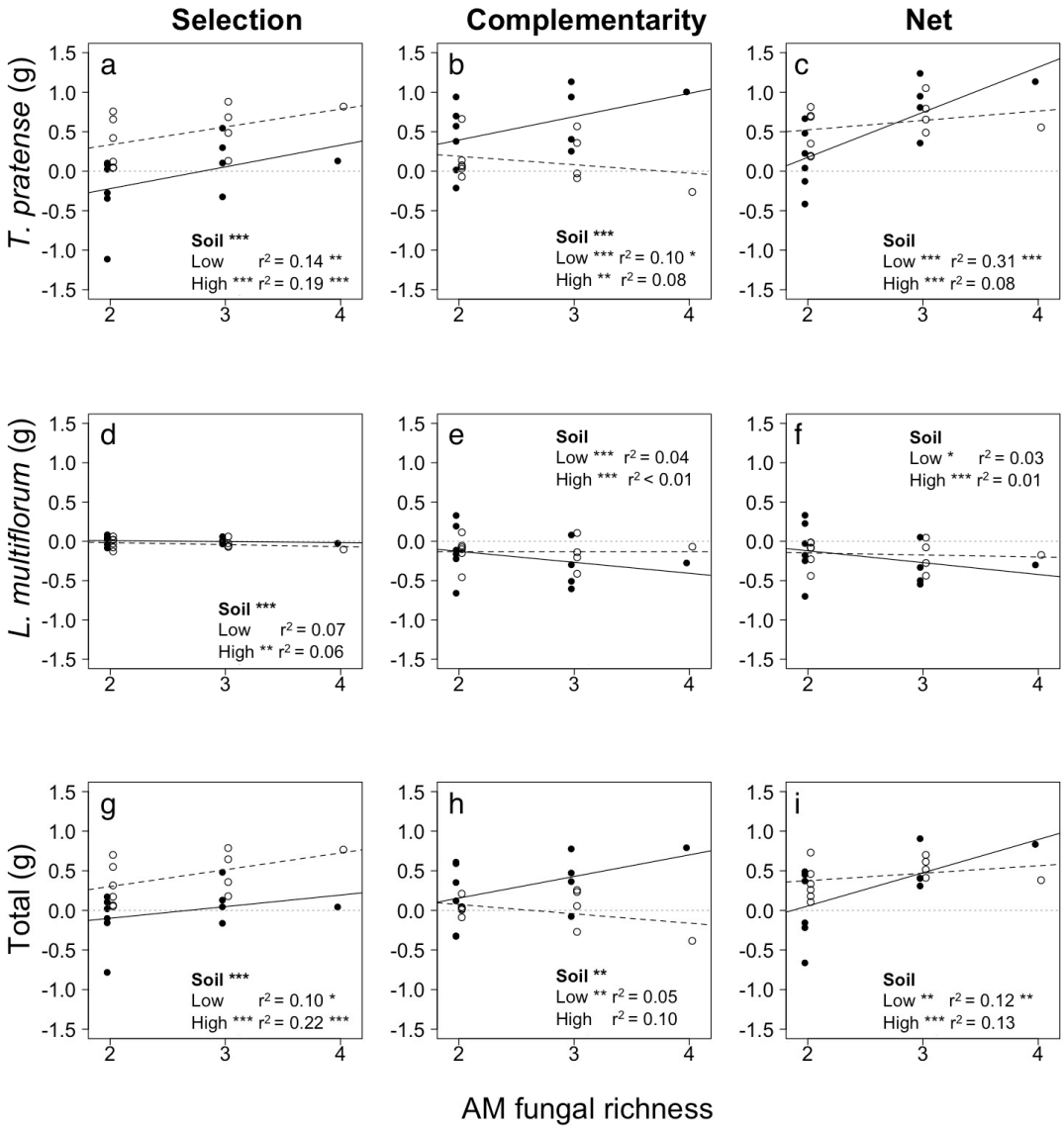

Figure 3. Selection, complementarity and net biodiversity effects of AM fungal richness on productivity of *T. pratense* (a–c), *L. multiflorum* (d–f) and their combined total (g–i) in both high-sand (open circles, dashed line) and low-sand (filled circles, solid line) soils. Initial AM fungal richness is shown on the x-axis. Significant differences in the overall effect between soils (Soil) and whether the effect in each soil differs from 0 is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significance levels adjacent to r^2 values indicate whether slopes differ significantly from 0.

DISCUSSION

It is known that selection and complementarity effects can enhance the performance of species-rich plant communities (Hooper *et al*. 2005). However, until now, the use additive partitioning (*sensu* Loreau & Hector 2001) to determine the role of selection and complementarity effects of soil microbial diversity on aboveground plant communities has not been tested. We observed a positive AM fungal richness-plant productivity relationships, similar to pervious studies (van der Heijden *et al*.

1998; Vogelsang *et al*. 2006; Maherali & Klironomos 2007), which were attributed to either a complementarity or selection effect depending on soil conditions. These biodiversity effects resulted from a range of interactions among AM fungi from facilitation to antagonism with consequences for aboveground plant productivity, particularly for *T. pratense*, the more symbiont-dependent plant species. Importantly, although the net biodiversity effect of AM fungi on plant biomass was similar in both soils, different mechanisms (either complementarity or selection effects) were responsible for the AM fungal diversity–plant productivity relationships. This illustrates how the overall effect of AM fungi on a plant community can be maintained under differing environmental conditions by a more AM fungal species-rich community despite the altered functioning of individual fungi.

Biodiversity effects of AM fungi on plant productivity

The overall effect of AM fungal richness on aboveground plant productivity primarily resulted from its strong positive effects on the legume *T. pratense*. In the less productive high-sand soil this effect was driven by the ability of *D. celata*, the most growth promoting AM fungus, to remain abundant within roots while the abundances of other co-inoculated AM fungi were reduced, indicating its ability to outcompete other AM fungi under these soil conditions. It is possible that the higher spore number in the *D. celata* inoculum favored its strong influence under these soil conditions. Nevertheless, this resulted in a significant and positive selection effect on *T. pratense,* and on total aboveground plant biomass.

The fact that in the high-sand soil, the AM fungus causing the selection effect also dominated the AM fungal mixture in terms of relative abundance is not surprising; yet not necessary for the selection effect to occur (Loreau & Hector 2001), as theoretically, a subdominant species could cause a positive selection effect. However, the parallel between the selection effect and the high abundance of the most beneficial AM fungus in mixtures is what Huston (1997) and Wardle (1999) predicted under the term “sampling effect” hypothesis: that improved productivity with increasing species richness is due to the greater probability of adding the most productive species that drives the functioning of mixtures. Complementarity among AM fungi was observed in both soils depending on AM fungal combination, such as the dual inoculation of *G. intraradices* and *G. claroideum*. Overall, in the high-sand soil a selection effect occurred more frequently, overriding the complementarity effect.

In contrast, in the more productive low-sand soil, more AM fungi-rich mixtures resulted in a greater aboveground plant response in *T. pratense* than any of the single species. Interestingly, the dual inoculation of *G. mosseae* with *D. celata* in the low-sand soil resulted in poorer *T. pratense* biomass production than the average of the two AM fungi mono-inoculated, demonstrating a negative selection effect and indicating an antagonistic interaction between the two fungal species. However, when more than these two species were present in a mixture, such a strong negative selection effect did not occur and the complementarity effect of AM fungal richness had a greater influence. This indicates *G. mosseae* was less effective at excluding the most productive species when challenged with a greater number of interspecific interactions and as a result, the complementarity effect increased with AM fungal richness driving the overall biodiversity effect.

Mechanisms of AM fungal coexistence and their biodiversity effects

We could verify the coexistence of AM fungi within the root system via qPCR, supporting a number of previous studies (e.g. Abbott & Robson 1984; Vandenkoornhuyse *et al.* 2002; Janoušková *et al.* 2009). The ability for AM fungi to coexist is a key factor as to whether biodiversity effects are driven by complementarity or selection effects, since the ability to coexist via niche segregation is a major component behind the two partitioned effects (Loreau & Hector 2001). In our experimental design, abiotic soil properties determined whether the functioning of AM fungal communities were driven by a single AM fungus (as observed for the high-sand soil) or whether multiple AM fungi coexisted (as observed in the low-sand soil) to influence the aboveground plant community. The substrate in the low-sand soil was perhaps more complex, thus allowing for better coexistence of a more functionally dissimilar community. The importance of such a link between resource complexity and the role of richness has been demonstrated by Jousset *et al*. (2011), where a more complex resource environment allowed for greater coexistence of functionally dissimilar *Pseudomonas fluorescens* genotypes, thus enhancing community functioning.

Whether a complementarity or selection effect is behind the AM fungal richness–plant productivity relationship can be due to numerous factors beyond abiotic characteristics, such as functional strategies of AM fungi. For example, similar to Jansa *et al*. (2008) and Janoušková *et al*. (2009), our results show that *G. intraradices* and *G. claroideum* are able to coexist and can facilitate greater benefits

to aboveground plant growth than either of the species inoculated individually; perhaps occurring via differences in foraging strategies (Jansa *et al*. 2005; Thonar *et al*. 2010). Additionally, spatial separation among AM fungi, such as via host preference in a plant polyculture, may maintain the functioning of a less competitive AM fungi (Bever *et al*. 2009) and thus enhance the complementarity effect of AM fungal communities. This potentially could explain the similarity between AM fungal monocultures and mixtures in their effect on plant productivity in some studies using a plant monoculture (e.g Jansa *et al*. 2008), but perhaps not others using a plant polyculture (e.g. Vogelsang *et al*. 2006).

Temporal variation among AM fungi has been observed (eg. Fitter & Merryweather 1998; Oehl *et al*. 2004) and thus their segregation in functioning through time is an additional mechanism by which AM fungi can differentially influence host responses. This could lead to a complementarity effect, however, may not be detected by sampling at a single time point, such as the case in our study. Detecting such an ebb and flow of colonization by different AM fungi through time that coincides with observable host effects will be an important feature for furthering the assessment of AM fungal biodiversity effects.

In general, it is difficult, if not impossible due to the seemingly limitless possibilities, to determine all the differences in functional and resource-based niches that each AM fungal species within a more AM fungal-rich community could occupy in order to influence whether a complementarity or selection effect drives the AM fungal richness–plant productivity relationship. However, since AM fungal taxa functional and life-history characteristics can be linked to phylogeny (Maherali & Klironomos 2007), the use of phylogenetic over- and under-dispersed AM fungal communities may give rise to understanding the different combinations of AM fungi within a community that best supports the aboveground plant community.

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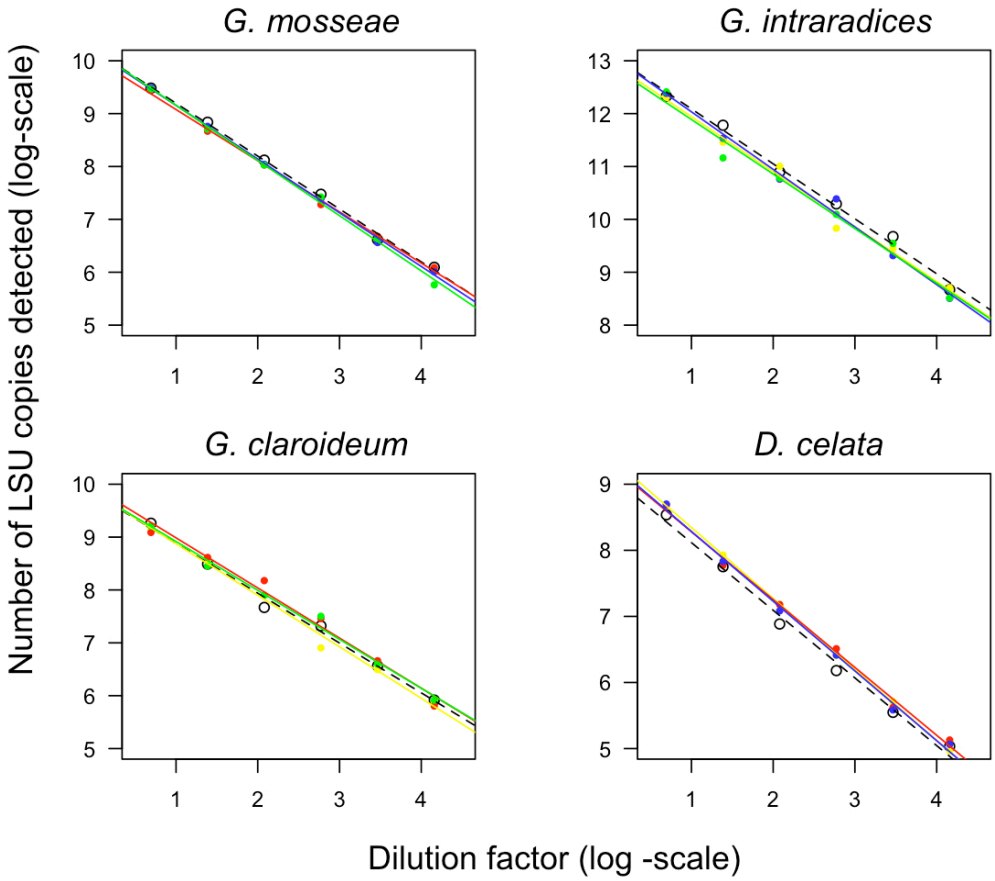
APPENDIX A: Soil characteristics and nutrient concentrations.

Soil characteristics and nutrient and mineral concentrations are shown for each soil. Water-soluble inorganic N (NO3– and NH4+) was determined with a Skalar segment flow analyzer.

Soil Characteristic	**'High-sand' Soil**	**'Low-sand' soil**
pH	7.7	7.5
Organic C	0.1 %	0.9 %
Clay	2.45 %	12.2 %
Silt	8.2 %	20.8 %
Sand	89.2 %	64.4 %
Plant available nutrients		
Water soluble NO_3- and NH_4+	20.5 $mg \cdot kg^{-1}$	50.5 $mg \cdot kg^{-1}$
P_2O_5 (CO_2-saturated water extracted)	0.71 $mg \cdot kg^{-1}$	0.32 $mg \cdot kg^{-1}$
K_2O (CO_2-saturated water extracted)	5.0 $mg \cdot kg^{-1}$	7.5 $mg \cdot kg^{-1}$
Ammonium acetate -extracted mineral nutrients		
Ca	7.02×10^3 $mg \cdot kg^{-1}$	4.26×10^3 $mg \cdot kg^{-1}$
P	33.5 $mg \cdot kg^{-1}$	17.7 $mg \cdot kg^{-1}$
K	2.85 $mg \cdot kg^{-1}$	24.4 $mg \cdot kg^{-1}$
Mg	96.6 $mg \cdot kg^{-1}$	160.9 $mg \cdot kg^{-1}$

APPENDIX B: Results for the test of interference by non-target DNA on the ability to detect target DNA.



Results for the test of interference by non-target DNA on the ability to detect target DNA. The target DNA, of a known concentration extracted from roots of AM fungal monocultures, of each AM fungus (*G. mosseae*, *G. intraradices*, *G. claroideum* and *D. celata*) was diluted in all PCR reactions with either water (open dots, dashed line), *G. mosseae* (yellow dots and lines), *G. intraradices* (red dots and lines), *G. claroideum* (blue dots and lines) or *D. celata* (green dots and lines) for all pair-wise combinations in the proportions 1:1, 1:3, 1:7, 1:15, 1:31 and 1:63 of target to non-target AM fungal DNA (dilution factor 2, 4, 8, 16, 32 and 64, respectively). The regression slopes did not differ among non-target treatments for the detection of either of the four target AM fungi (in all cases $F_{3,20} < 0.02$, $p > 0.99$) demonstrating that the abundance of non-target AM fungal DNA did not alter the ability for the primers and probes to detect their specific AM fungal target DNA.

APPENDIX C: ANOVA results for the number of LSU copies of each AM fungus. Two-factorial ANOVA tables with AM fungal treatment (AM) and soil treatment (Soil) as main sources of variation for the number of LSU copies detected of the four AM fungi. Significant *F* ratios are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. d.f = degrees of freedom, MS = mean squares. All AM fungal abundance data were log + 1 transformed prior to analyses.

		G. mosseae		*G. intraradices*		*G. claroideum*		*D. celata*	
	d.f.	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Block	1	12.6	3.54	28.4	5.25*	0.03	0.02	4.12	2.57
Soil	1	34.1	9.57**	91.7	17.0***	34.5	19.1***	1.22×10^2	76.3***
AM	7	32.2	9.02***	1.05×10^2	19.5***	17.1	9.45***	40.6	25.4***
Soil × AM	7	5.47	1.53	52.8	9.77***	13.6	7.52***	46.8	29.2***
Residual	79	3.57		5.41		1.81		1.60	

APPENDIX D: Means and standard errors for the number of LSU copies detected. Mean abundance (LSU copies per mg root) and standard errors of the mean for each of the four AM fungi in each soil and AM fungal treatment combination. The AM fungi present within a treatment are indicated by the letters: N = Non-mycorrhizal, M = *G. mosseae*, I = *G. intraradices*, C = *G. claroideum* and D = *D. celata*. NA = not assessed. The number of replicates in which each AM fungus was detected (n) is shown below each AM fungal combination. In samples where AM fungi were not detected their abundance was considered to be 0.

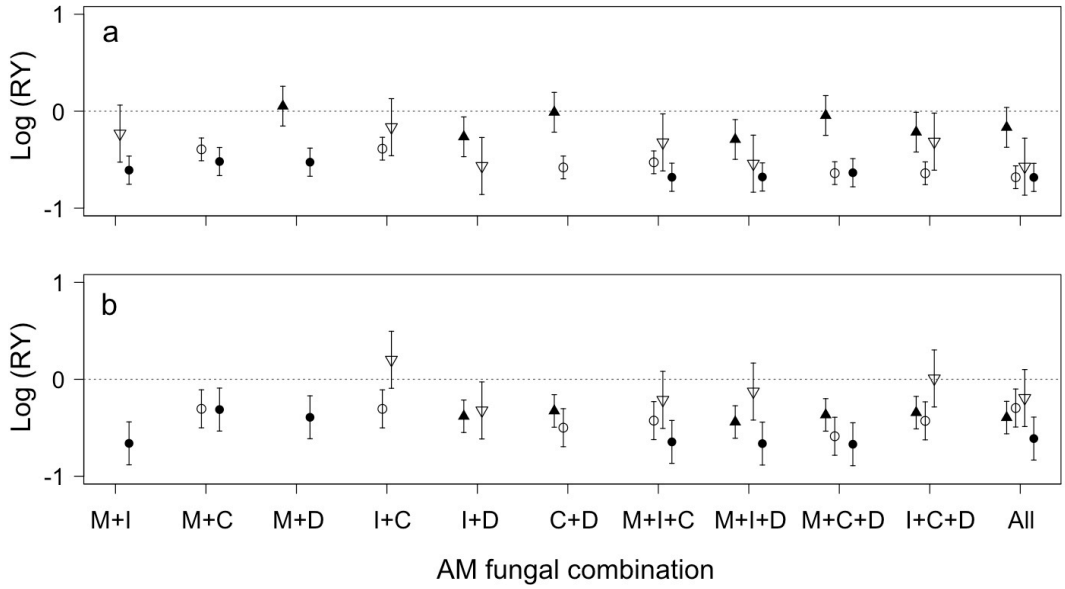
HIGH-SAND SOIL

AM fungal treatment	*G. mosseae* ($\times 10^3$)	*G. intraradices* ($\times 10^5$)	*G. claroideum* ($\times 10^4$)	*D. celata* ($\times 10^4$)
N	NA	NA	NA	NA
M (n=6)	258.6 (97.4)	NA	NA	NA
I (n=6)	NA	80.7 (5.18)	NA	NA
C (n=6)	NA	NA	30.9 (4.18)	NA
D (n=6)	NA	NA	NA	13.7 (3.77)
M+I (n=6, 6)	24.6 (14.4)	61.9 (29.2)	NA	NA
M+C (n=6, 6)	51.3 (16.7)	NA	11.1 (2.34)	NA
M+D (n=6, 6)	51.8 (25.3)	NA	NA	16.4 (4.25)
I+C (n=6, 6)	NA	64.1 (22.0)	12.1 (4.30)	NA
I+D (n=6, 6)	NA	11.2 (2.92)	NA	7.60 (1.46)
C+D (n=6, 6)	NA	NA	3.75 (0.95)	14.4 (3.27)
M+I+C (n=6, 6)	3.13 (2.47)	44.8 (21.2)	5.90 (2.34)	NA
M+I+D (n=6, 6, 6)	3.86 (2.51)	13.4 (2.54)	NA	7.14 (1.85)
M+C+D (n=6, 6, 6)	16.8 (12.1)	NA	1.74 (0.49)	12.9 (1.90)
I+C+D (n=6, 6, 6)	NA	39.0 (9.07)	1.68 (0.18)	8.54 (1.05)
M+I+C+D (n=5, 6, 4, 6)	2.70 (1.03)	10.6 (2.78)	0.37 (0.19)	9.77 (1.54)

APPENDIX D: *cont.*

	LOW-SAND SOIL			
AM fungal treatment	*G. mosseae* ($\times 10^3$)	*G. intraradices* ($\times 10^5$)	*G. claroideum* ($\times 10^4$)	*D. celata* ($\times 10^4$)
N	NA	NA	NA	NA
M (n=6)	34.2 (16.0)	NA	NA	NA
I (n=6)	NA	22.7 (5.08)	NA	NA
C (n=6)	NA	NA	29.3 (6.88)	NA
D (n=6)	NA	NA	NA	12.9 (2.43)
M+I (n=6, 0)	1.14 (0.57)	0.00 (0.00)	NA	NA
M+C (n=6, 6)	22.5 (13.3)	NA	15.7 (6.02)	NA
M+D (n=6, 0)	13.2 (5.00)	NA	NA	0.00 (0.00)
I+C (n=6, 6)	NA	34.6 (6.10)	14.6 (3.41)	NA
I+D (n=6, 6)	NA	11.1 (3.23)	NA	4.99 (1.38)
C+D (n=6, 6)	NA	NA	6.88 (3.17)	5.97 (1.38)
M+I+C (n=6, 5, 6)	1.69 (0.77)	17.8 (8.26)	9.81 (3.75)	NA
M+I+D (n=6, 6, 5)	1.04 (0.38)	19.06 (5.18)	NA	3.85 (0.91)
M+C+D (n=6, 6, 6)	0.82 (0.57)	NA	3.34 (1.03)	5.71 (2.61)
I+C+D (n=6, 6, 6)	NA	25.3 (6.74)	9.22 (2.41)	5.55 (1.10)
M+I+C+D (n=6, 6, 6, 6)	3.04 (1.47)	15.3 (2.89)	14.94 (3.39)	4.59 (0.81)

APPENDIX E. Relative abundance of each AM fungal species in mixtures.



Mean relative abundance of specific AM fungal species in mixture compared to their value in monoculture (log transformed means with 95 % confidence intervals are shown) in both high-sand (a) and low-sand (b) soils. The dashed line represents the abundance of each of the AM fungal species in their mono-inoculated treatments. Values above or below 0 indicate higher or lower abundance of an AM fungi in roots inoculated with an AM fungal mixture relative to roots where the AM fungi was mono-inoculated (*G. mosseae* = M = filled circles, *G. intraradices* = I = open triangles, *G. claroideum* = C = open circles and *D. celata* = D = filled triangles). Note: *G. intraradices* and *D. celata* were not detected in any of the replicates when inoculated together with *G. mosseae* (and no other AM fungal species) in the low-sand soil (b); their symbols are therefore absent in these treatments.

APPENDIX F: ANOVA results for biodiversity effects.

Summary of ANOVA results for the AM fungal biodiversity effects (Selection, Complementarity and their Net effect) on aboveground biomass of a) *T. pratense*, b) *L. multiflorum* and c) their combined total. Biodiversity effects were assessed for variation among soils (Soil), initial AM fungal richness (Richness) and among AM fungal treatments (AM treatment). The complementarity effect data for *T. pratense* and *L. multiflorum* were square-root transformed prior to analysis by multiplying -1 to negative data points before and after transformation to maintain direction. d.f. = degrees of freedom, MS = mean squares. Significance levels of the *F*-test are indicated as: **p*<0.05, ***p*<0.01, ****p*<0.001

		Selection		Complementarity		Net	
a) *T. pratense*	d.f.	MS	*F*	MS	*F*	MS	*F*
Block	1	0.09	1.88	1.76	7.78 **	1.69	8.14 **
Soil	1	9.20	187 ***	4.94	21.9 ***	0.34	1.65
Richness	1	3.56	72.2 ***	0.22	0.95	6.76	32.5 ***
AM treatment	9	0.64	13.0 ***	1.44	6.36 ***	0.49	2.38 *
Soil x Richness	1	0.03	0.70	2.11	9.34 **	2.89	13.9 ***
Soil x AM treatment	9	0.94	19.1 ***	0.37	1.63	0.79	3.77 ***
Residuals	109	0.05		0.23		0.21	
b) *L. multiflorum*							
Block	1	1.87×10^{-2}	7.02 **	1.27×10^{-1}	0.45	0.70×10^{-1}	0.34
Soil	1	3.45×10^{-2}	13.1 ***	0.09×10^{-1}	0.03	0.53×10^{-1}	0.26
Richness	1	1.98×10^{-2}	7.45 **	4.62×10^{-1}	1.64	4.42×10^{-1}	2.17
AM treatment	9	1.75×10^{-2}	6.57 ***	10.3×10^{-1}	3.63 ***	5.54×10^{-1}	2.71 **
Soil x Richness	1	0.26×10^{-2}	0.99	3.38×10^{-1}	1.20	2.24×10^{-1}	1.10
Soil x AM treatment	9	2.44×10^{-2}	9.17 ***	2.19×10^{-1}	0.77	2.54×10^{-1}	1.24
Residuals	109	0.27×10^{-2}		2.83×10^{-1}		2.04×10^{-1}	
c) Total							
Block	1	0.24	8.53 **	0.31	0.86	1.08	3.11
Soil	1	6.29	228 ***	2.86	8.06 **	0.67	1.93
Richness	1	1.82	66.0 ***	0.34	0.97	3.75	10.8 **
AM treatment	9	0.40	14.4 ***	0.75	2.11 *	0.30	0.86
Soil x Richness	1	0.06	2.18	2.16	6.10 *	1.50	4.35 *
Soil x AM treatment	9	0.57	20.6 ***	0.33	0.94	0.82	2.38 *
Residuals	109	0.03		0.35		0.35	

Chapter 3

EFFECTS OF LOCAL-SUBSTRATE AND PLANT-HOST PREFERENCES ON THE FUNCTIONING OF AN ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY

(A follow-up study on Chapter 2 - Unpublished)

ABSTRACT

The functioning of biodiversity in soils is currently a focal point in ecological research, as anthropogenic activity reduces fungal diversity and alters soil microbial community composition. Yet current knowledge as to the extent of its importance for maintaining ecosystem functions is lacking. Previous studies demonstrate increases in fungal diversity of symbiotic plant associated arbuscular mycorrhizal (AMF) can improve plant community productivity depending on abiotic substrate conditions. However, the mechanisms underlying the AMF diversity–plant community productivity relationship are unknown. Niche segregation among AMF species via different preferences in plant host and abiotic conditions are likely key features. We used quantitative PCR to detect the abundance of four AMF species in roots of a grass and a legume in mixture to assess whether AMF species differ in host and local substrate patch preferences. Additionally, we assess whether niche differentiation of AMF relates to plant productivity. We found plant host preference occured, primarily for the legume over the grass, and was influenced by the local substrate conditions. However, host preferences did not vary greatly among the four AMF species. Preferences for different local substrate patches did vary among AMF species. However, overall we found little evidence to suggest that a greater dissimilarity among these four AMF species in colonization preferences for host or substrate patch could relate to greater productivity of the plant community.

INTRODUCTION

The importance of diversity is recognized to be critical for maintaining ecosystem services (Hooper *et al*. 2005), stability (Tilman 1996; Yachi & Loreau 1999) and overall functioning (Zavaleta *et al*. 2010). Often studies manipulating species richness observe improved ecosystem functioning, such as greater productivity in plant communities, in more species rich communities (Balvanera *et al*. 2006). This effect of biodiversity on an ecosystem function can be partitioned into two main effects; the complementarity effect and the selection effect (Loreau & Hector 2001), of which the latter can be further partitioned into the dominance effect of a single species within a community (Fox 2005). The complementarity effect can be attributed to differences in niche partitioning among sympatric species (Loreau & Hector 2001). Differences among plant species in their ability to utilize different resource pools has previously been observed to be one mechanism by which species coexist via disparate niches (McKaine *et al*. 2002; Dimitrakopoulos & Schmid 2004; Harrison *et al*. 2007) and contribute to the complementarity and overyielding in plant species mixtures (Ashton *et al*. 2010).

Recently, biodiversity-ecosystem functioning research has turned to the relatively unexplored role of soil microbial communities and in particular the arbuscular mycorrhizal fungi (AMF). These fungi form symbiotic relationships with the majority of land plants (Smith & Read 2008) and their presence and diversity can influence the productivity and coexistence in plant communities (van der Heijden *et al*. 1998; Wagg *et al*. 2011ab). As in plant biodiversity studies, species richness of these plant-symbiotic fungi relationships have been shown to positively correspond with plant diversity and productivity (van der Heijden *et al*. 1998; Vogelsang *et al*. 2006; Maherali & Klironomos 2007) that can result from a complementarity effect of a more species rich AMF community, depending on soil abiotic conditions (Wagg *et al*. 2011b). This improved complementarity effect requires the various AMF within more species rich communities of AMF to occupy different niches (Loreau & Hector 2001).

AMF are a diverse group of plant symbionts known to operate among varying spatial and temporal environmental scales. For example, a number of studies have shown AMF differ in functional characteristics (Powell *et al*. 2009; Sikes *et al*. 2010; Thonar *et al*. 2010), temporal strategies (Fitter & Merryweather 1998; Pringle & Bever 2002), abiotic soil conditions (Johnson *et al*. 1992; Lekeberg *et al*. 2007, Oehl *et al*. 2010) and plant host preferences (Dhillion 1992; Bever *et al*. 1996; Bever 2002; Husband *et al*. 2002; Vandenkoornhuyse *et al*. 2003; Gollotte *et al*. 2004; Croll *et al*.

2008). All of these may be characteristics of niche spaces in which more species rich communities of AMF are able function complementarily to contribute to plant productivity (Wagg *et al*. 2011b).

Although positive effects of AMF diversity on plant productivity can occur and host specific AMF communities have been observed, there is little empirical evidence demonstrating AMF occupying disparate niches, such as via host preference, correspond to greater beneficial effects of an AMF community on aboveground productivity. Recently, however, evidence that a trade off between the ability to promote host growth and competition with sympatric AMF has been observed (Benett & Bever 2009). This would suggest avoidance of interspecific competition between AMF via niche segregation, such as spatial separation, could therefore enhance AMF coexistence and the communal functioning of AMF to provide greater beneficial effects to plant hosts (Bever *et al*. 2009). Such a spatial segregation among AMF within a natural environment could occur via host and substrate preference within a plant-AMF community.

The use of molecular tools to detect AMF community assembly within different co-occurring host plants has been shown to be a useful a useful tool for assessing host specificity (Sanders 2003). As a result, a number of studies have been able to illustrate the occurrence of AMF host preference (Croll *et al*. 2008; Sýkorová *et al*. 2007), but perhaps not in all soils (Schnoor *et al*. 2011). However, the influence of host preference within a plant-AMF community on the performace of the plant community has yet to be demonstrated. In this study we use real-time quantitative PCR (qPCR) to determine the abundance of four AMF in two plant hosts when co-inoculated to examine whether AMF show disparate host and substrate niche preferences as well as whether the niche disparity detected relates to aboveground plant productivity.

We hypothesize that if AMF differ in niche space (here plant host and substrate patches) then the greater disparity (less similarity) in AMF communities among these niches should relate to improved plant productivity. The study is broken into two paralleled experiments. The first re-examines the roots collected in Wagg *et al*. (2011b) to determine the AMF community assemblage within roots of either a grass (*Lolium multiflorum*) or a legume (*Trifolium pratense*) under two different soil conditions to assess the relationship between dissimilarity in AMF community assembly between the two hosts with plant community productivity and the previously determined biodiversity effects (see Wagg et al. 2011b). However, since

soil conditions determine the presence of AMF (Lekberg *et al*. 2007; Oehl *et al*. 2010) and how AMF function in a community context (Johnson 2010; Wagg *et al*. 2011ab), a second experiment was used to test for the dimension of AMF preference for hosts within three different substrate patches in which plants and AMF were able to freely colonize. These designs allow us to test not only for host by substrate niche preferences, but also tests for niche disparity among species and substrate patches as a mechanism by which sympatric AMF can communally improve primary productivity.

METHODS

Inocula, substrate, and seedling preparation

We used four different AMF that commonly co-occur in Swiss agricultural grasslands: *Glomus intraradices* (isolate BEG 21), *G. mosseae* (isolate BEG161), *G. claroideum* (isolate JJ132) and *Diversispora celata* (isolate FACE 234). Each of the four AMF inocula was prepared by culturing on *Plantago lanceolata* for 6 months in sterilized 1:9 ratio by volume of quartz sand to field soil substrate. Inocula cultures were dried after 6 months of propagation and roots were cut into < 5 cm pieces and homogenously mixed prior to use. All four inocula were found to be viable sources of AMF propagules and readily colonize both hosts (see Wagg *et al*. 2011a).

The growing conditions for the culturing of AMF inocula, as well as for both experiments described below, consisted of 16 h / 25 °C days, under natural light maintained above 300 W/m^2 by 400 W high pressure sodium lights, and 8 h / 16 °C nights and received dH_2O to maintain soil moisture at approximately 20 % by weight. The field soil used in the substrate mixtures in both experiments and inocula preparation was collected from an agricultural grassland located at the ART Reckenholz research station in Zürich, Switzerland (047° 42’ 74’’ N, 008° 51’ 78’’ E). All substrates used in both experiments and for the preparation of inocula were sterilized by autoclaving for 99 min at 121 °C (see below for specific substrate preparation details for each experiment).

Seeds of *Trifolium pratense* var. Milvus and four *Lolium multiflorum* var. Daxus were surface sterilized by slow agitation in 1.25 % sodium hypochlorite for 5 minutes, followed by rinsing in dH_2O. Seeds were then germinated on 1.5% water agar before transplanting into pots. Seedlings not surviving transplant were replaced within two weeks of the initial planting.

To ensure rhizobia bacterial association established in pots, 1 L of field soil from the above-mentioned natural grassland was wet-sieved with approximately 5 L of dH_2O through series with the smallest being 11 μm in mesh size. Ten ml of the microbial wash per 1 L of pot volume was added to each pot in both experiments. The formation of root nodules by *T. pratense* was observed in all pots and no AMF colonization of roots was observed in any of pots where AMF were not inoculated.

Experiment 1 design

Plant roots were collected from a previous study by Wagg *et al*. (2011b). In brief, the treatment consisted of 1 L round pots filled with a mixture of field soil, sieved through a 0.5 cm mesh, and quartz sand in either a ratio of 1:4 or 4:1 by volume of soil to sand, respectively. This created pots with either a “high-sand” or “low-sand” soil treatment (see Table 1 for soil characteristics). In each pot 12.5 ml of each of the four AMF inocula was homogenously mixed throughout the soil substrate. Four *T. pratense* and four *L. multiflorum* were grown for 25 weeks. Each soil type was replicated 6 times for a total of 12 pots. Pots were evenly divided and randomly positioned within two greenhouses. Shoots were cut 4 cm above the soil surface after 9 and 16 weeks to restrict competition for light and simulate mowing. After 25 weeks plants were harvested directly at the soil surface all plant shoots were dried at 70 °C and the biomass of both plant species was recorded and the data from all harvests were pooled.

Experiment 2 design

Pots, 3 L in volume, were sectioned vertically into 3 equal parts with each part filled with 1 L either quartz sand, field soil or organic matter which was previously pasteurized via autoclaving. The organic matter consisted of ground woody debris, plant litter and peat (see Table 1 for details) and the field soil was collected from the same location as mentioned above. These three substrates were chosen to maximize differences in abiotic substrate conditions. No barriers were present between substrate patches so that roots and fungi could freely colonize each patch. In each of the three 1 L substrate patches, 12.5 ml of each of the four AMF inocula were mixed homogenously throughout. In each substrate patch 3 *T. pratense* and 3 *L. multiflorum* were transplanted and evenly spaced for a total of 18 plants per pot. In addition a control treatment was inoculated with 50 ml of *P. lanceloata* roots and substrate free of AMF propagules. This control inoculum was grown under the same

conditions mentioned above for the preparation of the AMF inocula. The inoculated and control pots were replicated 7 times and randomly distributed throughout a single greenhouse and grown for 12 weeks under the above-mentioned conditions. Plants were harvested at the soil surface, dried and the biomass of both plant species recorded as described in experiment 1.

Table 1. Soil mineral and nutrient composition for each of the soil / substrate types used

	Experiment 1		Experiment 2		
Soil Characteristic	**High-sand**	**Low-sand**	**Sand**	**Soil**	**Organic**
pH	7.7	7.5	7.0	6.5	8.0
Plant available nutrients ($mg \cdot kg^{-1}$)					
Water soluble NO_3- and NH_4+	20.5	50.5	2.70	11.77	207.1
P_2O_5 (CO_2-saturated water extracted)	0.71	0.32	2.99	0.71	15.3
K_2O (CO_2-saturated water extracted)	5.0	7.5	3.0	8.0	475
Ammonium acetate-extracted mineral nutrients ($mg \cdot kg^{-1}$)					
Ca ($\times 10^3$)	7.02	4.26	6.69	1.51	25.2
P	33.5	17.7	48.9	6.4	11.5×10^2
K	2.85	24.4	15.7	53.0	68.2×10^2
Mg	96.6	160.9	61.7	145	21.9×10^2

Processing of roots

At the final harvest in both experiments, roots were cut into small fragments (1- 2 cm). Roots of *L. multiflorum* and *T. pratense* were separated under a stereo binocular microscope. Roots of the two plant species were distinguished by morphological characteristics where *T. pratense* roots are thicker, yellower in colour and having root nodules with lateral roots originating from a primary root. *Lolium multiflorum* roots were identified as pale white, without nodules and longer root hairs. Roots were lyophilized and a portion, of which the weight was recorded (20 - 25 mg), was sampled for DNA extraction using the Qiagen DNeasy plant mini kit following manufacturer recommendations for the purification of total DNA from plant tissue (Qiagen Sciences, Germantown, Maryland, USA).

Molecular analysis

The nuclear large ribosomal subunit (LSU) of each AMF was amplified by real-time qPCR using primers and hydrolysis probes specific to each of the four AMF. The hydrolysis probe details, primer sequences and qPCR cycle program can be found elsewhere (Wagg *et al*. 2011a; Thonar *et al*. 2012). The qPCR reactions were carried out using the LightCycler 2.0 (Roche Applied Science, Rotkreuz, Switzerland). The cycle threshold values of each qPCR reaction were used to determine the number of LSU copies per mg of lyophilized root following the calibrated equations for each AMF outlined in Wagg *et al*. (2011a).

Data analysis

All four AMF were detected in every pot where they were inoculated except in experiment 2, where *G. claroideum* was not detected in either host in both the organic and sand patches in a single pot and thus considered 0 abundance for determining proportional abundance of the AMF in each of the plant host as well as for determining community similarity. Since in experiment 2 not all plants survived, the biomass of each of the host plants is expressed as the biomass per individual plant surviving at the end of the experiment for both studies. The number of plants surviving was also added to all models as a covariate.

Host preference of each AMF was assessed by the number of LSU copies detected in the roots of each host plant in each soil and soil patch using a mixed effects model and the restricted maximum log-likelihood method with host plant, soil or substrate patch and their interaction, as fixed sources of variation. The data from experiment 1 required a hierarchical error of the greenhouse block in which plants were grown and the pot within blocks in which the two host plants co-occurred to be added as random effects. For experiment 2, random effects were added as a hierarchical error with the substrate patch in which the two host plants were grown together within the pot that contained the three soil patches. The LSU abundance data was log+1 transformed in experiment 1 and by the power of 0.2 in experiment 2, as indicated by Box-Cox transformations and which best improved homoscedasticity in the data.

Host plant responses to AMF inoculation were calculated as the difference in biomass between the fungal inoculated and non-inoculated treatments. The plant responses to AMF inoculation were also assessed in a mixed effects model with the same fixed and random effects as mentioned above for each experiment.

Additionally, the addition of the proportional abundance of each AMF detected within each of the host roots and their interactions with host and soil effects were fit to the models by the stepwise addition of terms to detect any AMF host preference – host productivity relationships. Note that the proportional abundance is relative to the total abundance of an AMF detected within a pot, such that a proportional abundance of 1 indicates the fungus was only detected within that particular host or soil patch.

The similarity in the AMF community colonizing roots of host plants and soils was determined as the percent similarity (*PS*) calculated as; $PS = \sum \min(p_i, p_j),$

where p_i is the proportion of a single AMF detected within a host in a given substrate and p_j is the proportion of that same AMF within the alternative host. This measure is ideal for proportional data and is less influenced by a particularly abundant species than other measures (Smith *et al*. 1990). In order to determine whether the similarity between the communities colonizing roots of the two plant hosts differed between soils and substrate patches in both experiments was assessed by mixed effect model with soil as a fixed effect and random effects being the same as the analyses on plant host responses mentioned above. Additionally, the similarity between pairs of substrate patches within each pot in experiment 2 was also assessed with the substrate patch pair (sand-soil, soil-organic, and sand-organic) as fixed effects and the pot within which the substrate patches co-occurred as a random effect.

In order to determine whether AMF community similarity between the two host plants corresponded to the average biomass response of the two plant hosts, the average response of the two host plants was assessed by a mixed effects model with the similarity between hosts and soils for experiment 1. In addition, the biodiversity effects (determined previously in Wagg *et al*. 2011b) were also assessed for relationships to the AMF community similarity between host plants in experiment 1 after first accounting for differences between soil types within the model. The average biomass response of the two host plants in experiment 2 was assessed with substrate patch and community similarity as well as their interaction as fixed effects. The relationship the average biomass response of the plants and the AMF community similarity among substrate patches was also assessed in a separate model. Random effects were added to the model in the same hieratical structure as mentioned above.

RESULTS

Host and soil patch preferences

In experiment 1, only *G. claroideum* differed in abundance between soil treatments ($F_{1,9}$=29.6, p<0.001), while *G. mosseae*, *G. intraradices*, and *D. celata* showed no overall difference ($F_{1,9}$ = 1.51, p = 25; $F_{1,9}$ = 1.72, p = 0.22 and $F_{1,9}$ > 0.01, p=0.93 respectively; see Fig. 1). An overall difference in the abundance of *G. intraradices*, *G. claroideum* and *D. celata* was detected between the two host plants ($F_{1,11}$=6.74, p=0.02; $F_{1,10}$=28.5, p<0.001, $F_{1,10}$=8.33, p=0.02, respectively) demonstrating a host preference by these fungi for *T. pratense* over *L. multiflorum* (Fig. 1). However, in the case of *G. claroideum* and *D. celata* the difference between host plants was only detected in the high-sand soil (soil x host interaction $F_{1,10}$=11.8, p=0.01 and $F_{1,10}$=5.71, p=0.04, respectively). No difference in the abundance of *G. mosseae* was detected ($F_{1,11}$=1.45, p=0.25).

In experiment 2 *G. mosseae*, *G. claroideum* and *D. celata* all differed in abundance among substrate patches ($F_{2,12}$=5.41, p=0.02; $F_{2,12}$=5.29, p<0.02 and $F_{2,12}$ = 7.66, p=0.01, respectively) indicating a substrate patch preference for root colonization (Fig. 1). Only *G. intraradices* did not differ in abundance among soil patches ($F_{2,12}$=0.41, p=0.67). None of the four AMF exhibited an overall difference in abundance between plant hosts (*G. mosseae* $F_{1,19}$=2.25, p=0.15; *G. intraradices* $F_{1,17}$=3.36, p=0.08; *G. claroideum* $F_{1,19}$=0.93, p=0.35; *D. celata* $F_{1,19}$ = 3.90, p=0.06). However, *G. intraradices* was more abundantly detected in *T. pratense* roots in the organic substrate only (Fig. 1; host x soil interaction $F_{2,17}$=3.72, p=0.04).

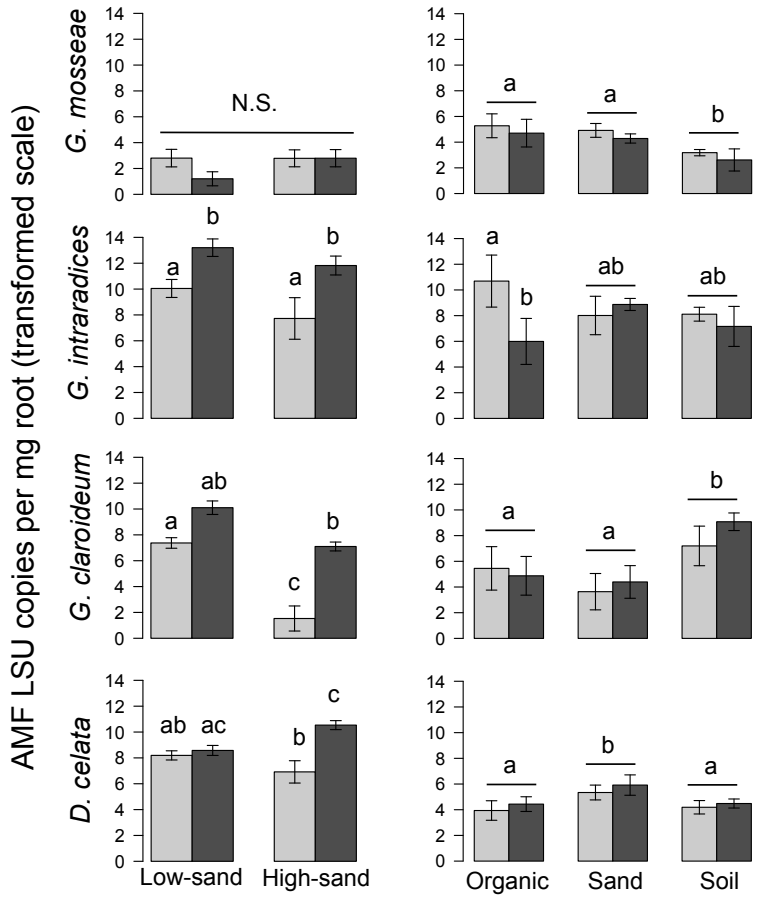


Figure 1. The mean and standard error of the number of AMF LSU copies per mg of root detected in *L. multiflorum* (light bars) and *T. pratense* (dark bars) of the model estimates are shown for both experiment 1 (left column) and experiment 2 (right column). Different letters indicate where the source of variation in the model was found to be significant. The y-axis is scaled to the transformed data used in analyses; log transformation for experiment 1 and the power of 0.2 for experiment 2.

Host performance

In experiment 1, *L. multiflorum* individuals in the absence of AMF produced 618.3 mg (SE = 40.7) in the high-sand soil and 1777.9 mg (SE = 98.2) in the low-sand soil. In presence of AMF, the growth of *L. multiflorum* was reduced resulting in an overall negative response to the inoculated AMF community (see Fig. 2a). In contrast, *T. pratense* growth was 73.3 mg (SE = 6.63) per individual in the high-sand soil and 48.0 mg (SE = 4.50) in the low-sand soil in the absence of AMF, but was greatly improved by AMF inoculation in both soils (see Fig. 2a). As a result, the variation in the response of the aboveground plant community was largely due to the differences between the two plant hosts ($F_{1,11} = 171$, $p<0.001$), where biomass was greatly improved in *T. pratense* and marginally depressed in *L. multiflorum* (see Wagg *et al*. 2011b for further details). The proportion of each of the AMF detected in

the roots of either of the two hosts and their interactions with soil treatment could not significantly improve the explanatory significance of the model. This reflects a greater abundance of an AMF species in one host over the other host did not relate to any changes in the growth response of the plant host (Fig. 2a).

In experiment 2, *T. pratense* growth in the absence of AMF was 104.5 mg (SE=30.0) in the organic substrate, 130.3 mg (SE=34.4) in the sand substrate and 213.7 mg (SE =65.3) in the soil substrate. The growth of *L. multiflorum* in the absence of AMF was 701.6 mg (SE=114.4) in the organic substrate, 610.5 mg (SE=69.6) in the sand substrate and 1251.1 mg (SE =252.5) in the soil substrate. Overall, aboveground plant biomass response to AMF inoculation did not differ among substrate patches ($F_{2,10}$=3.16, p=0.09), but did differ between host plants ($F_{1,13}$=104.1, p<0.001) and among a host plant by substrate patch interaction ($F_{2,13}$=12.1, p=0.001). This resulted from *T. pratense* benefiting the most from AMF inoculation in both the soil and sand substrates (an increase of 125.8 mg, SE=24.0, and 97.3 mg, SE=36.0, respectively), which differed from the negative responses in *L. multiflorum* in both these substrate patches (-340.7 mg, SE=57.6, and -80.0 mg, SE=32.1, respectively). In the organic substrate *T. pratense* and *L. multiflorum* responses to AMF inoculation did not differ (23.1 mg, SE=25.2, and -83.5 mg, SE=64.6, respectively). The proportion of *G. claroideum* within a host root system did however differ in effect on the host response between the two plants ($F_{2,13}$=6.37, p=0.01). This resulted from *T. pratense* biomass not relating to the proportional abundance of any AMF detected within its roots (Fig. 2b) while *L. multiflorum* did show a positive relationship to the proportion of *G. claroideum* (Fig. 2c).

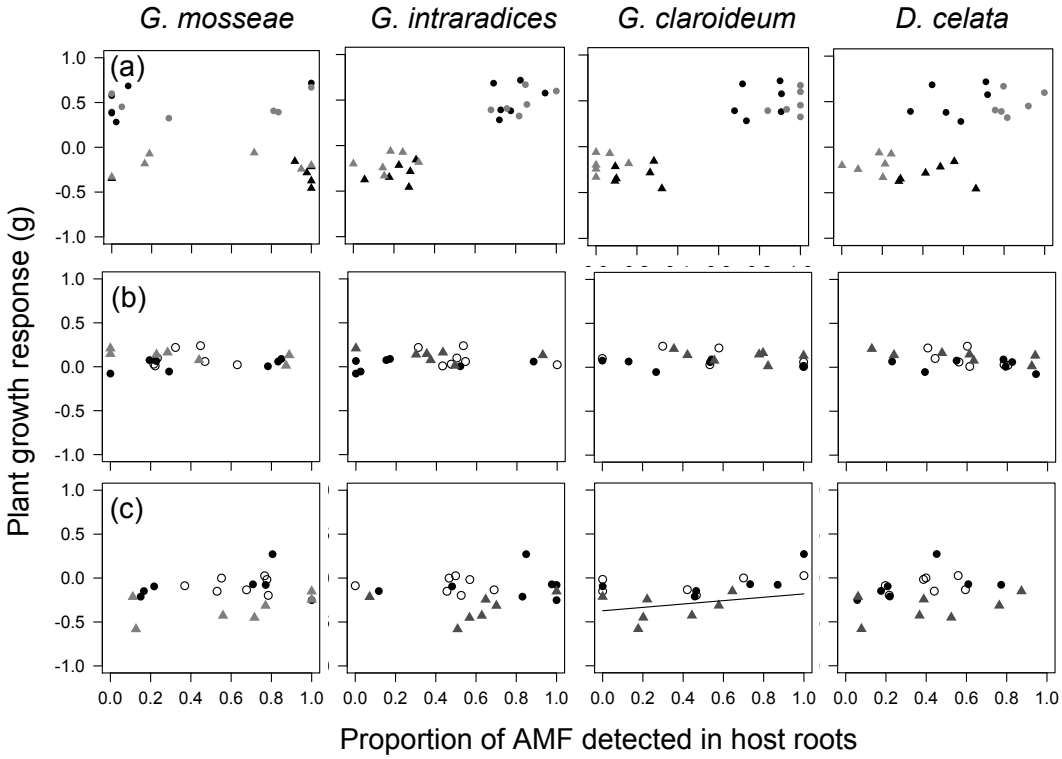


Figure 2. Relationship between plant growth response to AMF inoculation and the proportional abundance of an AMF within the plants roots are depicted for (a) *T. pratense* (circles) and *L. multiflorum* (triangles) in experiment 1 in both the high-sand (grey) and low-sand (black) soils. For experiment 2 relationships are shown separately for (b) *T. pratense* and (c) *L. multiflorum* (c) in the sand patch (open circles), organic patch (black dots) and soil patch (triangles). Significant relationships are indicated by the regression line and are derived from the model after accounting for differences among soil patches and host species.

Fungal community similarities and plant community response

AMF community assembly was highly similar between the two host plants in both experiments. In experiment 1, AMF community similarity between host plants in the low-sand soil was 88.7 % (SE = 1.7) and did not differ significantly ($F_{1,9}$=2.55, p=0.15) from that in the high-sand soil which was 73.5 % (SE = 9.4). In experiment 2, AMF community similarity between the two host plants also did not differ significantly ($F_{2,12}$=2.32, p=0.14) within the three substrate patches: organic = 70.9% (SE=9.9), sand = 80.8% (SE = 5.9), soil = 84.6% (SE = 5.5). However, the overall community similarity varied between substrate patches ($F_{2,11}$=4.18, p=0.04). The AMF communities colonizing roots were most similar between the organic and sand substrates (90.9 %, SE =0.82) and were least similar between the organic and soil substrates (81.3 %, SE=3.52), while the sand and soil substrates were 83.9 % (SE=4.12) similar.

The community similarity between host plants did not relate to the overall plant community growth response to AMF inoculation in experiment 1 ($F_{1,8}$ = 0.20, p=0.67; Fig. 3a) and also showed no relationship to any of the biodiversity effects in experiment 1 (determined in Wagg *et al.* 2011b); complementarity effect: $F_{1,8}$ = 0.02, p=0.89, selection effect: $F_{1,8}$ = 4.01, p=0.08 and the net effect: $F_{1,8}$ = 0.20, p=0.67. In experiment 2, the overall plant community growth response to AMF inoculation varied in relationships to the percent similarity in AMF communities between host plants among the different substrate patches (substrate patch x similarity interaction: $F_{2,8}$=4.68, p<0.05); where there was a marginally significant negative relationship in the soil substrate which differed from the relationships in the other substrate patches (Fig. 3b). There was no overall effect of AMF community similarity between host plants ($F_{1,8}$=0.17, p=0.69), nor was the similarity among substrate patches related to aboveground plant response to AMF inoculation ($F_{1,12}$=0.26, p=0.62; Fig. 3c).

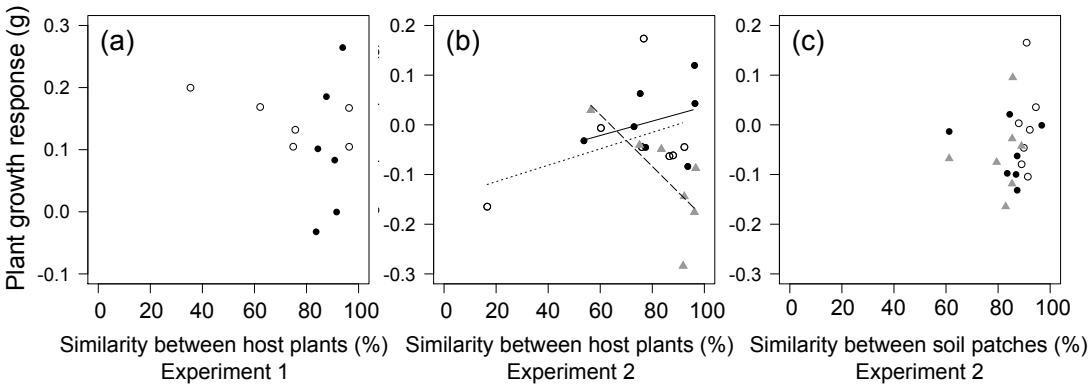


Figure 3. The relationship for the average growth response of *T. pratense* and *L. multiflorum* individuals to the community similarity between host plants for both (a) experiment 1 (high sand = open circles; low sand = filled circles) and (b and c) experiment 2 (organic patch = open circles and dotted line (r^2 = 0.18, p = 0.34); sand patch = filled circles and solid line (r^2 = 0.10, p = 0.48); soil patch = grey triangles and dashed line (r^2 = 0.54, p = 0.06)).

DISCUSSION

Although differences in host preference within an AMF community may be a mechanism to reduce niche overlap in sympatric AMF, little has been done to assess the potential ecological importance of AMF host preference. Our results suggest AMF niche disparity via host preference is not a strong mechanism for improving aboveground plant community productivity. We found in general, similarity in the AMF community colonizing the two plants was largely dependant on the substrate in which the two host plants occurred. Thus indicating substrate conditions can exert stronger controls on the community assembly of AMF within plant roots than the identity of the

host plant. In addition, relationships between the AMF community similarity colonizing plant hosts with aboveground plant productivity also depended upon the localized substrate patch. This would suggest that the plant growth responses to this AMF community is not driven by host plant preferences, but perhaps other functional niches the fungi occupy within localized substrate patches. Additionally, the responsiveness of the plant species used may have also played a role, where the plant community responses were largely driven by AMF dependant plant *T. pratense*. Further assessments of plant community productivity to host preference disparity among AMF should include a greater richness of plant species beyond the use of single AMF responsive and non-responsive plants used here.

Only in the soil substrate in the second experiment did we find a negative relationship between the AMF community similarity between host plants and the aboveground plant community. Although marginally non-significant, this could suggest a greater dissimilarity in the AMF community colonizing the two host plants can correspond to greater benefits for the productivity of the plant community. Intriguingly, this was the substrate patch with the lowest level of plant available P and total extractable P (see Table 1); the primary soil resource AMF are well know to provide their hosts (Smith & Read 2008). It is conceivable that P was limiting to plant productivity within this substrate and the greater AMF community dissimilarity between the two host plants allowed for reduced intraradical competition between fungi for host resources. The reduction in interspecific competition via spatial segregation in host plants may therefore enhance the ability of the community of AMF to improve symbiotic functioning of the overall AMF community (Bennett & Bever 2009; Bever *et al*. 2009). However, further evidence for is required as this trend was not consistent; perhaps due to results being temporally dependant.

Mycorrhizal preference and host benefits

The degree to which host preference occurred seemed to have minimal effects on host plants. We were not able to detect consistent increases in plant responses to the degree of AMF host preference, except in the case of *G. claroideum* detected within *L. multiflorum* roots. Intriguingly in this particular case, it seems *G. claroideum* preferentially colonized *L. multiflorum* resulted in an increase in AMF benefits to its productivity. This parallels the result of Wagg *et al*. (2011a) where this same fungus was most abundantly detected in a *L. multiflorum* monoculture providing

further support for a host preference – productivity relationship in this mycorrhizal association.

The influence of soil conditions on AMF

The fact we observed substrate dependant AMF host preferences supports previous work illustrating abiotic soil properties control AMF community structure and functioning (Lekberg *et al*. 2007; Johnson 2010: Wagg *et al*. 2011a,b). When the AMF community was provided with the two plant hosts in a homogenous substrate in experiment 1, the majority of the AMF showed a host preference for the more symbiont dependant plant (*T. pratense)*. This result parallels previous studies demonstrating the occurrence of plant host preference (Bever *et al*. 1996; Bever 2002a; Dhillion 1992; Husband *et al*. 2002; Vandenkoornhuyse *et al*. 2003; Croll *et al*. 2008). However, when provided the same plant hosts within different substrate patches in experiment 2 the same AMF showed a greater preference to certain soil patches than host plants. This would demonstrate abiotic soil conditions drive the colonization abilities of these fungi.

Connections between environmental conditions and the functioning of AMF host preference or specificity have been observed in host parasite interactions (Wolinska *et al*. 2009; Mostowy & Engelstädter 2010) and therefore may like-wise drive the AMF-host preference and relationships. Our results support this concept that the realized niche of an AMF species is determined more by the abiotic environment than host compatibility. This seems particularly evident considering most, if not all, AMF species can colonize all AMF compatible plants (Smith & Read 2008). The control of the abiotic substrate conditions on the association between AMF and plant host species may contribute to the stochastic nature of AMF community assembly in nature (Drumbrell *et al*. 2009; Lekberg *et al*. 2011) and may also help explain why AMF host preference is not always observed (eg. Schnoor *et al*. 2011). Thus, future studies will need to focus on the variations in AMF extradical mycelial colonization among substrate patches as a niche segregation strategy in AMF communities, particularly since they have been shown to differ in soil foraging strategies (Thonar *et al*. 2010). This will be a key feature for understanding the mechanisms behind functional complementarity in AMF communities within the natural heterogeneous soil ecosystems.

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Chapter 4

SOIL BIODIVERSITY LOSS RESULTS IN THE DECLINE OF MULTIPLE ECOSYSTEM FUNCTIONS

(Unpublished)

ABSTRACT

The current condition of global biodiversity loss has become a major concern as mounting evidences indicates it will have repercussions for the ecosystem processes modern society depends upon. However, the assessment of biodiversity loss on ecosystem processes are largely based upon aboveground organisms while a highly diverse and complex ecosystem belowground, known to be involved in numerous ecosystem processes, is often over looked. Here we assess the consequences of soil biodiversity loss on multiple key ecosystem functions within a model grassland ecosystem through a twice-repeated experiment. Soil biodiversity loss resulted in reduced plant diversity, reduced decomposition of organic material, reduced nitrogen cycling and increased nutrient leaching. As a result ecosystem multifunctionality declined with increasing destruction of soil biodiversity. Shifts in the majority of these ecosystem functions were marginal with the loss of organisms at higher levels of diversity, but declined dramatically as soil biodiversity was suppressed toward the minimum achievable limit. These findings demonstrate high levels of soil biodiversity are required for optimal ecosystem functioning.

INTRODUCTION

Global biodiversity loss has become a major concern for maintaining the services ecosystems provide society (Rockstrom *et al*. 2009; Hooper *et al*. 2012). The loss of biodiversity in aboveground terrestrial ecosystems has consequences for maintaining functioning of multiple ecosystem processes (Hector & Bagchi 2007; Zavaleta *et al*. 2010; Maestre *et al*. 2012), yet current knowledge of biodiversity loss from soils and the ecosystem level consequences is in its infancy. The various components of soil biota are known to be instrumental in numerous ecosystem processes, from nutrient cycling to plant coexistence and productivity, both directly and indirectly (Brussaard *et al*. 1997; van der Heijden *et al*. 2008; Bardgett & Wardle 2010). Soil communities are highly diverse on extremely smaller scales than their counterpart communities aboveground and may constitute the majority of biodiversity in terrestrial ecosystems (Fitter *et al*. 2005; Parker 2010). Yet, this unseen majority in ecosystems has often been overlooked (Allison & Martiny 2008; van der Heijden *et al*. 2008).

The concern for understanding the consequences of soil biodiversity loss stems from mounting evidence that anthropogenic activities, such as agricultural intensification, cause reduced microbial and faunal abundance in soils and alters the composition of these communities (Helgason *et al*. 1998; Mäder *et al*. 2002; De Vries *et al*. 2006; Verbruggen *et al*. 2010). These changes likely have repercussions for the functioning of numerous ecosystem processes such as resource cycling between above and belowground ecosystems (Wardle *et al*. 2004; De Vries *et al*. 2012). Furthermore, the depletion and simplification of soil biodiversity may compromise soil fertility through increased soil erosion, leaching, and reduced turnover of plant available nutrients in arable soils (Briones *et al*. 1998; Kibblewhite *et al*. 2008). These concerns stem from mounting evidence that suggests the diversity of specific groups, such as faunal or microbial organisms, aid in the maintainence of plant diversity and plant productivity (van der Heijden *et al*. 1998; Bradford *et al*. 2002; Bonkowski & Roy 2005; Maherali & Klironomos 2007), soil nutrient retention (Bradford *et al*. 2002; Bonkowski & Roy 2005; Dybzinski *et al*. 2008; Eisenhauer *et al*. 2010; van der Heijden 2010), and decomposition (Bradford *et al*. 2002; Eisenhauer *et al*. 2010). Such evidence indicates numerous ecosystems processes to be a function of soil biodiversity. However, not all studies consistently demonstrate greater diversity in belowground communities consistently improves ecosystem functions, and frequently a specific ecosystem function reaches an asymptotic maximum at low levels of

diversity (eg. Heemsbergen *et al*. 2004; Setälä & McLean 2004; Wertz *et al*. 2006). Consequently, the extent of overall soil biodiversity loss that can simultaneously affect multiple ecosystem process uncertain and generated much debate.

It has been discussed whether there is a high level of redundancy in soil communities such that many soil organisms have little to no influence on an ecosystem function (Wardle 1999; Setälä *et al*. 2005; Allison & Martiny 2008). For instance, small changes in soil biodiveristy may have no effect in soil ecosystems with high levels of soil biodiversity, but may result in reduced ecosystem functioning in ecosystems already poor in biodiversity (Wall 2006). Coarser scale reductions of soil biodiversity, such as loss or simplification of guilds of soil organisms, would result in reduced overall functional diversity within the soil ecosystem and likely have much stronger negative consequences on the functioning and resilience of the soil ecosystem (Hunt & Wall 2002; Wall 2006; Griffiths *et al*. 2000; De Vries *et al*. 2012). A cohesive understanding of soil biodiversity required to achieve optimal functioning of multiple ecosystem processes may thus be hampered by assessments of single groups of organisms or ecosystem process, since soil organisms and the processes they drive within natural ecosystems are likely highly interconnected (Kibblewhite *et al*. 2008). As a result, empirical evidence for a comprehensive understanding of the mechanistic importance of soil biodiversity for overall ecosystem functioning is currently missing (Wall *et al*. 2010). Here, we investigated whether the degradation of soil biodiversity holds negative consequences for multiple ecosystem functions.

METHODS

Microcosms

Forty-eight experimental grassland microcosms were established under sterile conditions in closed growth chambers (see Appendix A). In order to avoid any greenhouse borne microbial contamination from the outside, incoming pressurized air was filtered through a hydrophobic filter with a pore size of 0.2 µm (Millex®-FG_{50}; Millipore Corporation, Billerica, USA) and incoming water was filtered through a hydrophilic filter with a 0.22 µm pore size (Millex®-GP_{50}; Millipore Corporation, Billerica, USA). The microcosms were assembled, inoculated, and planted within a laminar flow hood to minimize the possibility of contamination. All parts used for the microcosms were sterilized by autoclaving for 30 min at 120 °C, with the exception of the Plexiglas tops and the PVC bottoms (see Appendix A). The bottom and top of the microcosms (which deform when autoclaved) were sterilized by submersing in 0.5%

sodium hypochlorite for 20 - 30 minutes then in 70% Ethanol with Tween 20 for a few minutes and air-dried within a Laminar flow hood.

Substrate and inocula

Each microcosm was filled with a 1 cm layer of < 1 cm diameter quartz stones at the bottom and topped with a 0.5 mm propyltex mesh (Sefar AG, Heiden, Switzerland). Each microcosm was filled with 5.5 kg (dry mass) of a 1:1 quartz sand: field soil mix that was sterilized by autoclaving for 90 min at 120 °C under pressure. The field soil was collected from a grassland located at the Agroscope Reckenholz research station in Zürich, Switzerland (47° 25' 38.71'' N, 8° 31' 3.91'' E) and was sieved through a 5 mm mesh before use.

Different soil biodiversity treatments were created by wet sieving 250 g of fresh 5 mm sieved field soil collected, from the same above mentioned field, with a total volume of 1L dH_2O through a series of decreasing mesh sizes so that each inoculum consisted of the soil community < 5000 μm, < 250 μm, < 50 μm, < 10 μm (Watmann paper No. 1), and sterile inocula (created by autoclaving for 90 min at 120 °C). By using different sieves it is possible to exclude soil organisms with variable body size (eg. Bradford et al. 2002). All soil material not passing through the sieves was collected, autoclaved, and added to back into the inoculum in order to control for differences that may occur via sieving out larger soil components. Each of the 5 inocola were mixed throughout the substrate in each microcosm and replicated 8 times for a total of 40 microcosms. Two additional replicates of each soil inoculated soil substrate were created and used for analyzing soil characteristics. No differences in soil substrate characteristics were detected among soil community treatments (see Appendix B).

In order to measure N turnover, two 6 cm x 6 cm litterbags made of the 0.5 mm propyltex mesh were filled with 0.18 mg of dried *Lolium multiflorum* shoots, previously labelled with ^{15}N ($d^{15}N=17.2 \times 10^2$; 2.72 % N) were inserted into each microcosms just below the soil surface. The ability of *L. multiflorum* plants to then reabsorb and incorporate ^{15}N from this organic source again into aboveground biomass is defined here as N turnover. Additionally, litter decomposition was assessed as the remaining dry mass of the litter at the end of the experiment and expressed as a percentage of the original litter mass.

Plant community

The plant community within each microcosm consisted of 40 individual plants comprising 10 species characteristic for many Swiss grasslands: legumes (5 *Trifolium pratense* and 5 *Lotus corniculatus*), grasses (4 *Lolium multiflorum*, 5 *Poa annua* and 5 *Festuca pratensis*) and forbs (3 *Prunella vulgaris*, 2 *Senecio jacobea*, 4 *Plantago lanceolata*, 3 *Achillea millefolium*, and 4 *Capsella bursa-pastoris*). Seeds of each species were surface sterilized by gentle agitation in 50 ml of 2.5 % sodium hypochlorite with a few drops of Tween 20, for 10 min followed by three rinses with 1 L sterilized dH_2O. Seeds were then evenly spaced onto 1% water-agar media in Petri dishes until germination. Since germination times varied among species, seed preparation was staggered so that each species germination date coincided. Germinated seedlings (presence of cotyledon(s)) were transplanted into 40 predetermined evenly spaced positions at random in each microcosm in a laminar flow chamber to avoid microbial contamination.

The plant and soil communities established and developed under natural light with additional lightning provided by 400-W high-pressure sodium lamps to maintain 16 h / 25 °C days and 8 h / 16 °C nights with a light level above 300 W/m^2. Microcosms were watered with dH_2O every 48 – 72 hours to maintain soil moisture in the range of 10–20 % by weight (65 - 85 % water holding capacity of the soil). The experiment was repeated twice; for a growth period of 14 weeks during the summer of 2010 (Experiment 1) and for a growth period of 24 weeks during the spring-summer of 2011 with an intermediate harvest at 12 weeks (Experiment 2; see below for modifications to Experiment 2).

Data collection

Microcosms were harvested by replicate blocks at each harvest. Prior to harvest microcosms were watered to 10-15 % above water holding capacity and the first 500 ml of leachate that percolated through the soil column was collected from a small outlet at the bottom of each microcosm (see Appendix A). Collected leachate was analyzed for nutrient concentrations (PO_4, NO_3, NH_4) as described in van der Heijden (2010). Total P was determined using a spectrophotometer (Helios Gamma, Thermo Scientific, Digitana AG, Switzerland) following the molybdate blue method (Murphy & Riley 1962). Leachate samples from one of the 8 replicate blocks were compromised during collection and 5 samples are therefore absent in the data set. Plants were harvested at the soil surface (with the exception of the 12 week harvest

in the second run; see below) and plant dry weight was determined for each plant species and the number of individuals harvested per species counted. Plant diversity (Shanon's index) in each microcosm was determined using the biomass per number of individuals harvest as the abundance of each plant species. At the end of Experiment 1 and Experiment 2, litterbags were collected, rinsed clean of soil, dried and remaining litter dry weight determined. Four soil cores, 2.5 cm in diameter, were taken to the depth of the substrate, mixed carefully and frozen at -20 °C for further processing. A 500 mg sample of each soil sample was then used for DNA extraction using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Switzerland) following the manufactures instructions. Roots were washed free of soil, the fresh weight recorded, and frozen at -20 °C at the time of harvest until further processing.

Modifications and additional measurements in the second experimental trial

The experiment was repeated in 2011 using the same substrate, plant community, preparation of material, and growth conditions previously mentioned. Some modifications were added to the experimental design: plants were grown for a total of 24 weeks instead of 14 weeks with an intermediate harvest at 12 weeks by clipping plants approximately 5 cm above the soil surface. This was performed in order to simulate hay making or grazing, commonly employed at natural grasslands. At the time of the 12-week harvest, three soils samples were taken using 2.5 cm diameter cores following the procedure described above. Before harvesting the external surfaces of the microcosms were sterilized by submersing in 70% ethanol prior to opening and all work was done within the Laminar flow hood to reduce the possibility of outside contamination. To further refine the detection of soil communities at smaller filter scales an additional treatment of soil inocula was added, namely a filtering through 25 μm mesh. Thus, experiment 2 consisted of 6 treatments (I: < 5000 μm, II: < 250 μm, III: 50 μm, IV: < 25 μm, V: < 10 μm, and VI: 0 μm / sterilized soil). Each treatment was replicated in 8 blocks as before with the exception of treatment VI that had 10 replicates (50 microcosms in total). To improve the ability to measure litter decomposition, 1 mg of ^{15}N only labelled (Note: concentration not yet determined) *Lolium multiflorum* leaf litter was added per litterbag and two litterbags per microcosm were submerged in the soil as before.

In order to improve the ability to detect nutrients leached and measure N_2O emissions (see below), microcosms were given a 50 ml nutrient solution corresponding with a fertilizer application of 60 kg N per ha and 10 kg P per ha.

(containing 778 mM KNO_3, 59 mM KH_2PO_4,1 mM $MgSO_4$ *$7H_2O$, 2 mM $CaCl_2$*$2H_2O$, 50 μM KCl, 25 μM H_3BO_3, 2 μM $MnSO_4$*H_2O, 2 μM $ZnSO_4$ *$7H_2O$, 0.5 μM $CuSO_4$*$5H_2O$, 0.5 μM Na_2MoO_4*$2H_2O$ diluted in 440 ml of dH_2O) and watered to water holding capacity 6 hours before the first N_2O measurement. N_2O production was measured by cycling microcosm air through a LI-820 CO_2 Gas Analyzer (LI-COR Biosciences, Lincoln, US) and subsequently to a TEI 46c automated N_2O analyzer (Thermo Fisher Scientific, Waltham, US) for a period of 10 minutes. This was done 3 times per day prior to harvest at 24 weeks (see Appendix C). The data was summed over this period and the total N_2O emitted was assessed as an ecosystem function. At the time of harvest, microcosms were again watered just above water holding capacity and leachate collected as before.

Molecular assessment of soil communities

Bacterial and fungal community composition was determined using ribosomal internal spacer analysis (RISA). Soil extracted DNA was quantified using the Quant-iT™ PicoGreen® (Molecular Probes, Eugene, OR) on a Cary Eclipse Fluorescence Spectrophotometer, diluted to 10 ng / μl and used as DNA template in PCR reactions. Bacterial community profiles were generated using the primers bRISArev and bRISAfor (FAM-labelled) using the cycling conditions and reagent concentrations outlined in (Hartmann *et al*. 2005; see reference for these primers). For fungal community profiles the primers fRISArev and fRISAfor (FAM-labelled) were used following the reagent concentration and cycling conditions outlined in Schneider *et al*. (2010). Two μl of the PCR products were mixed with 12 μl HiDi-Formamid and 0.2 μl MapMarker® 1000 (BioVentures, Murfreesboro, TN) as the size standard and subject to fragment analysis using an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Run conditions were set to injection times of 30 s at 1.5 kV and 10 s with a run time of 3000 s at 10kV. Unambiguous peaks of amplified DNA fragments were characterized based on their relative migration units using GenMarker 1.5 genotyping software (SoftGenetics LLC, State College, PA) and used as operational taxonomic units (OTU). Peak intensities of the OTUs were scored as with a threshold value of 20. OTU peak intensities were converted to proportions of the total peak intensity sum in each sample. Prior to analyses, bacterial and fungal OTU data matrices were standardized by z-score transformations of each OTU column (mean = 0, SD = 1) and each row in the data set normalized; such that the sum of squares of each row = 1. Note: this data provide a preliminary assessment of

the effect of filter treatments on soil biodiversity. A more detailed assessment of various groups of soil organisms is currently being performed and not part presented in this PhD dissertation.

Data analysis

All data manipulations and statistics were done using R software (version 2.13.0; The R Foundation for Statistical Computing 2011) and packages 'nlme', 'lme4' and 'AICcmodavg' for assessing mixed effect models; package 'vegan' for diversity measures, ordinations, and assessment of Euclidean distances in OTU composition. 'Significance' was considered at a type I error of $\alpha < 5$ %. All statistical models included plant mortality (number of individuals) as a covariate. Replicate blocks within harvest date were random effects in all mixed effect models.

Assessment of soil communities

Fungal and bacterial OTU richness were assessed separately for each experimental trial (data was pooled for harvest in the second experimental trial). OTU richness, as well as OTU diversity (Shannon index *H'*, using normalized relative peak intensity as a surrogate for abundance), was assessed in a mixed effects model with soil biodiversity treatment (filter size as a factor) as the fixed effect. Means were then contrasted against the most complete soil inoculum treatment (5000 μm) in order to detect changes in the OTU communities in the various soil biodiversity treatments. The average Euclidean distance among OTUs was calculated separately for each experimental trial and harvest to visually summarize community changes in sequential soil treatments (see Appendix D). Euclidean distance matrices were further assessed by MANOVA using the function 'adonis', in package 'vegan', and the structure visualized using constrained redundancy analyses (RDA) using filter size treatments as the predictor of OTU community structure for 200 permutations (see Appendix E).

Assessment of ecosystem characteristics and multifunctionality

Data for each ecosystem characteristic (listed in Table 1) were combined for both experimental trials. This was done by first centring and normalizing the data for each variable by z-transformations in order to adjust for differences in overall means and variance in measures between trials and harvests. Leachate and N_2O emission data were multiplied by -1 (inverted around the 0 mean) to maintain directional

change with other ecosystem functions from a desirable state (ie. when more nutrients were leached the ecosystem function declines). Raw means and standard errors of the means for each of these characteristics and individual plant species are presented in Appendix C, G, and H (see Appendix F for the productivity of individual plant species). In addition, each ecosystem function was combined into a single ecosystem multifunctionality index (*sensu* Maestre *et al*. 2012) using the average of the z- transformed data of each of the ecosystem functions collected at the final harvest of each experiment highlighted in Table 1 (all characteristics excluding grass, forb and legume productivity data, since these data comprise the net productivity and plant diversity measures).

In order to improve homoscedasticity in models, litter decomposition, N turnover, legume and forb biomass required log transformation (calculated as y=log(y+1+|min(y)| to deal with negative values) and N_2O emitted and all leachate data required square root transformation (using absolute values with negative values being multiplied by -1 post transformation to maintain direction). The data were assessed separately in mixed effect models with the inoculated soil biodiversity treatments (as a factor) as a fixed effect and treatment means were contrasted to the full soil inoculum treatment (filter size of 5000 μm). The data were scaled above zero (such that the minimum value = 0) and were divided by mean of the full soil treatment (filter size of 5000 μm) in order to visualize the proportional change in each ecosystem characteristic along the gradient of soil biodiversity treatments (filter size).

Additionally, each ecosystem function was assessed by multiple linear regression in mixed effect models using the log-scale filter size of the inocula treatments (continuous variable), bacterial OTU richness, and fungal OTU richness as predictors with terms fitted in order by which they explained the greatest amount of variation indicated by the sum of squares (presented in Table 1). All possible models comprising the various combinations of these three independent variables (including a null model with all three predictors absent) were compared by the second-order Akaike information criterion (AICc). Finally, linear regression was used compare relationships in the multifunctionality index to the three characteristics of soil biodiversity (filter size, bacterial and fungal OTU richness) between the two experimental trials. Correlations among all ecosystem functions, multifunctionality index, and characteristics of the soil biodiversity treatment (filter size, bacterial and fungal OTU richness) were considered and are presented in Appendix I.

Table 1. Effects of soil biodiversity treatment (filter size as factor) and soil community characteristics (filter size as a continuous variable, bacterial and fungal OTU richness) on ecosystem characteristics. Values for the regression models (filter size, bacterial OTU, fungal OTU) are the sum of squares with all terms present in order by which they explain the greatest amount of variation. Numbers preceding values indicate rank of the model in which the term was included based on minimizing AICc values. Only the top three models are presented and the difference in AICc values from the best model (terms with superscript 1) are shown. Note in some cases the null model was highest ranked and thus no term is ranked with a 1). Terms that explained the greatest amount of variation and were included in the best model are bolded. The multifunctionality index is comprised of the ecosystem functions highlighted in grey. df = degrees of freedom (numerator, denominator), ns = P > 0.05, * = P<0.05, ** = P < 0.01, *** P < 0.001. § = data comprised from final harvest only of both trials, † = 2010 final harvest data only, †† = 2011 final harvest data only.

	Soil biodiversity treatment		Regression Models				
Ecosystem characteristic	**df**	**F value**	**Filter size**	**Bacterial OTU**	**Fungal OTU**	**ΔAICc model 2**	**ΔAICc model 3**
Grasses	5, 110	49.3 ***	3.97 [1,3]	0.13 [3]	**64.9** [1,2,3]	1.42	13.3
Forbes	5, 110	42.3 ***	**8.13** [1,3]	< 0.001 [3]	0.81 [1,2,3]	3.04	13.4
Legumes	5, 110	97.5 ***	**13.1** [1,2,3]	0.46 [2]	0.05 [3]	4.30	11.3
Net Primary Productivity	5, 110	13.6 ***	2.14 [2]	0.34	**12.4** [1,2]	3.61	8.56
Plant Diversity	5, 110	55.7 ***	**62.3** [1,2,3]	1.27 [2]	6.78 [1,2]	7.58	9.93
Litter Decomposition §	5, 68	13.6 ***	0.33 [2,3]	0.04	**3.41** [1,3]	0.55	4.94
N turnover †	4, 27	10.2 ***	0.02 [2]	< 0.001 [3]	**4.78** [1,2,3]	8.11	12.2
N_2O emission††	5, 36	3.36 *	0.003 [3]	0.004	0.097 [2]	5.69	5.84
NH_4 leached§	5, 64	3.64 *	0.31 [3]	3.20	4.14 [2]	1.50	2.83
NO_3 leached §	5, 64	1.98 ns	0.22 [3]	0.30	1.89 [2]	5.58	6.34
PO_4 leached §	5, 64	1.31 ns	< 0.001 [2]	0.23	0.39 [3]	7.32	8.70
Total P leached §	5, 64	7.74 ***	**13.7** [1,3]	0.34	0.86 [2,3]	5.75	6.76
Multifunctionality index	5, 110	12.6 ***	**7.60** [1,2,3]	0.93 [2]	0.51 [3]	7.49	8.85

RESULTS

We successfully established a soil biodiversity gradient by filtering out living soil through different mesh sizes. Overall, bacterial and fungal OTU richness varied among soil biodiversity treatments ($F_{1,110} = 20.2$, $P < 0.001$ and $F_{1,110} = 94.4$, $P < 0.001$, respectively) and was greatest in treatments inoculated with the most complete soil fraction (< 5000 μm) and decreased with declining filter size, being 59 % and 80 % lower, in the treatment with sterile soil, respectively for bacterial and fungal OTU richness (Fig. 1). Moreover, microbial community structure varied significantly among treatments in both trials for both bacterial (MANOVA $F_{4,35} = 1.88$, $F_{5,49} = 1.69$ and, $F_{5,49} = 1.61$) and fungal OTU communities (MANOVA $F_{4,35} = 2.02$, $F_{5,49} = 2.06$ and, $F_{5,49} = 1.88$) at all three sampling time points 2010 trial, 2011 at 12 weeks and, 24 weeks respectively (all $P < 0.01$; see Appendix D and E). Typically, both fungal and bacterial OTU communities detected in treatments soil fractions size 0, 10 and 5000 μm respectively were most distinct (Appendix E) and the fungal and bacterial OTU communities became increasingly dissimilar from the most 'complete' soil community inoculated (organisms < 5000 μm) with increasing filtering of the soil inocula (Appendix D). Although, bacterial and fungal richness was lowest in the treatment with sterile soil, several bacterial and fungal OTUs were present, reflecting bacteria and fungi that entered the system as seed borne microbes, intact DNA of non-living microbes, or perhaps of air borne contamination during microcosm set up.

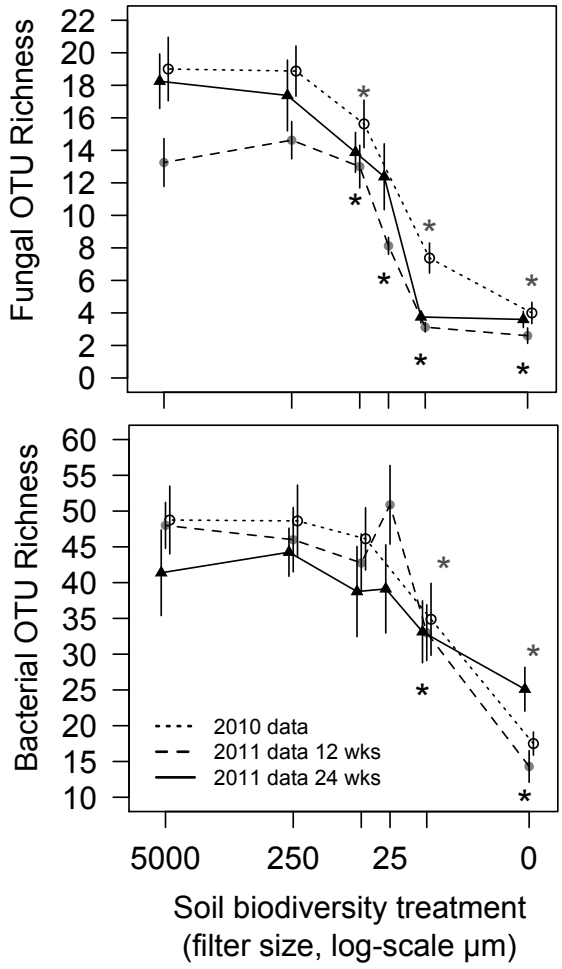

Figure 1. Fungal and bacterial OTU richness detected across the gradient of soil biodiversity treatments. Error bars are 1 standard error of the mean above and below means. Treatments that differed significantly from the most complete soil treatment (5000 µm) are indicated by astricts (*) above the means in grey for the 2010 data and in bold below means for the 2011 data (pooled for both harvests).

Effect on plant communities

Soil biodiversity had a positive effect on biomass production in forbs and legumes while negatively influencing grasses (Table 1; Fig. 2). Legumes and forbs; respectively contributing 11 – 24 % and 33 – 36 % of total primary productivity in the treatment with highest soil biodiversity and 1.1 – 2.3 % and 6.6 – 14.1 % in the treatment with sterilized inocula and the lowest soil biodiversity. This resulted in a dominance of grasses contributing 84-92 % of total primary productivity when inoculated with sterilized soil. As a consequence, plant diversity was highest in treatments with the highest soil biodiversity and declined with the removal of consecutive fractions of living soil (Fig. 2). The effect of soil biodiversity loss on the plant community thus resulted in a simplified plant community, primarily found to be a

function of filter size and fungal richness (Table 1; Fig. 2). Not all forb and grass species responded similarly to reductions in soil biodiversity. Specifically, the forb *S. jacobea* and the grass *F. pratensis* did not show any consistent trend in growth across the soil community gradient (Appendix F). Interestingly the non-mycorrhizal plant *C. bursa-pastovis* also did not follow the typical productivity trend as the majority of the forbs, but demonstrated greatest productivity at intermediate degradation of the soil community.

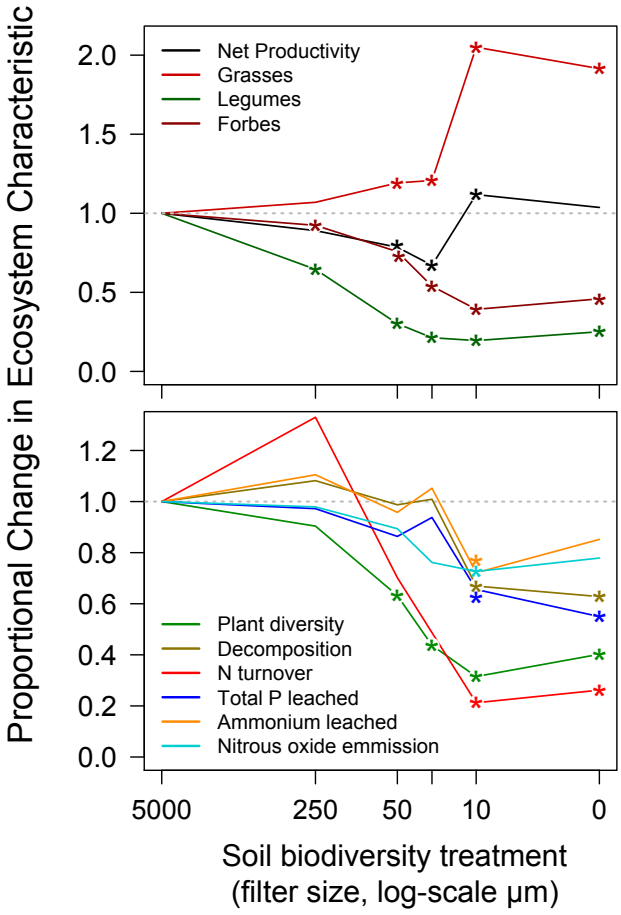

Figure 2. Proportional changes in ecosystem characteristics from the most complete fraction. Astrics (*) indicate where means differed significantly ($P < 0.05$) from the complete soil fraction (5000 µm; indicated by grey dotted line = 1). Only ecosystem characteristics varying significantly among the soil biodiversity treatments are shown (see Table 1 for complete list of investigate parameters).

Effect on litter decomposition and nutrients lost

Turnover of plant available N, litter decomposition, N_2O gas emission, and leaching of total P and NH_4 were significantly affected by the soil biodiversity treatments (Table 1). Litter decomposition increased with soil biodiversity and was primarily a function of fungal OTU richness in the soil and filter size (Table 1). Similarly, N turnover was declined with decreasing soil biodiversity and was primarily attributed to the fungal OTU richness (Table 1; Fig. 2). Moreover, total P leaching, NH_4 leaching, and N_2O losses were greater at low levels of soil biodiversity (Table 1; Fig 2). In the 2010 trial concentrations of leached nutrients were frequently below detection, particularly for PO_4 and NO_3, while in the 2011 trial PO_4 and NO_3 were exceptionally high (see Appendix H). This is due to the addition of these nutrients prior to leachate collection in the 2011 trial and perhaps a high level of depletion of these nutrients from the soil in the 2010 trial. Overall, both NO_3 and PO_4 did not vary

significantly along the soil biodiversity gradient or among the filtered soil treatments (Table 1). However, NO_3 and net productivity followed the same trends along the soil biodiversity gradient and were highly correlated (see Appendix I).

Ecosystem mutlifunctionality declined with the suppression of soil biodiversity (Fig. 3). Multifunctionality declined linearly in both experimental trials with decreasing filter size of the fraction of living soil added to each microcosm (Fig. 3), bacterial OTU richness (Fig. 3), and fungal OTU richness (Fig. 3). Although most of the ecosystem functions were best explained by the fungal community detected in the soils, the filtered size of the soil fraction explained the greatest proportion of variation in the multifunctionality index (Table 1).

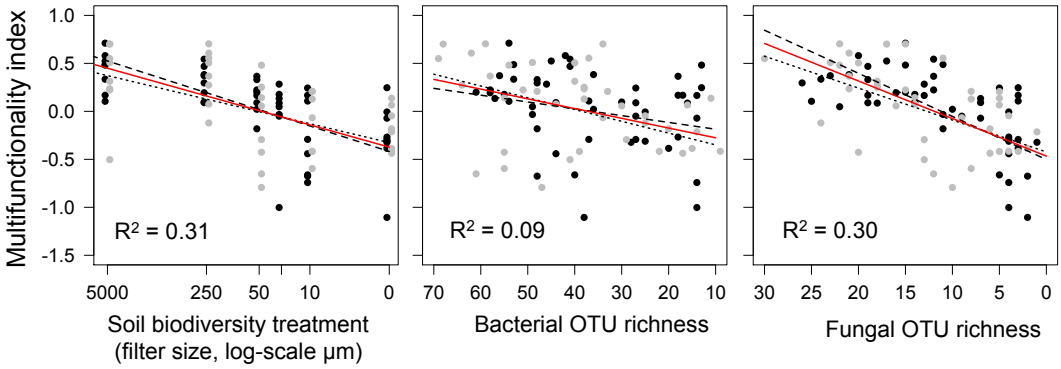

Figure 3. Response in ecosystem multifuncitonality to soil biodiversity indicators for trial 1 (grey dots, dotted line), trial 2 (black dotes, dashed line) and both trials combined (redline). R^2 values refer to the overall relationship highlighted in red. The multifunctionality index is the consolidation of ecosystem functions in Table 1. In all cases relationships did not differ between 2010 and 2011 data.

DISCUSSION

This work provides the first comprehensive view of the consequences of soil biodiversity loss on multiple key ecosystem functions. Seven out of 9 ecosystem functions investigated were negatively affected by soil biodiversity loss. As a consequence, ecosystem multifunctionality, declined linearly with the depletion of soil biodiversity. As in aboveground ecosystems, this illustrates high levels of soil biodiversity are required to achieve optimal ecosystem functioning (Zavaleta *et al*. 2010). The shifts in specific ecosystem functions began slowly with the removal of soil organisms eliminated at larger filter sizes, and dropped dramatically when the soil biotic community was degraded toward the extreme. Changes in litter decomposition, N turnover, and net productivity were best explained by the richness of the detected fungal community. Plant diversity, total P leached, and the overall

multifunctionality of the microcosoms were best explained by the filter size, reflecting organisms removed based on size, including soil invertebrates, mycorrhizal fungi, and protozoa (Bradford *et al*. 2002). Changes in the composition and abundance of these organisms are not reflected in the fungal and bacterial OTU data. Considering the inocula filtration gradient used reflects losses of soil functional guilds, our results may support the concept that reductions in soil functional diversity occurs with the removal of functional guilds of organisms, from macrofauna to microbial, and reaches a tipping point at which ecosystem functioning declines rapidly (Hunt & Wal 2002; Wal 2006).

Plant diversity and productivity

Our results show that a reduction in soil biodiversity altered the plant community composition and resulted in suppressed plant diversity. Grasses increased in abundance with reduced soil biodiversity while the abundance of forbs and legumes declined. These changes in plant community composition are likely a result of both direct and indirect mechanisms. For example, many legume and forb species are known to depend upon arbuscular mycorrhizal fungi for acquiring soil resources (eg. van der Heijden *et al*. 1998). Thus the suppression of this functional guild of soil organisms may reduce the competitive ability of forbs and legumes with grasses (Marler *et al*. 1999; Wagg *et al*. 2011a,b) with the decline in soil biodiversity below 50 μm. This result is in agreement with past studies demonstrating that plant diversity is reduced when mycorrhizal fungi are absent (van der Heijden *et al*. 1998; Wagg *et al*. 2011a). Conversely, the abundance of soil-borne pathogens can alter the productivity and competitive ability of specific species and alter the contributions of these plant species to the net productivity (e.g. van der Putten *et al*. 1993; Maron *et al*. 2011). Fungal pathogens may have contributed to the productivity of the grasses in our study since their productivity was best explained by the fungal OTU richness.

Nutrient cycling and retention

Soil organisms are well known to mediate soil resources via mineralization and decomposition of organic material (Heemsbergen *et al*. 2004; Setälä & McLean 2004; Bonkowski & Roy 2005). This functioning of soil biodiversity is a key characteristic in shaping and maintaining aboveground communities (Bardgett & Wardle 2010; Scherber *et al*. 2010). Our results demonstrate that the loss of soil biodiversity not only reduced decomposition of organic matter, but also limited the

liberation of plant available nitrogen from organic material. Although, both fungi and bacteria are involved in this process, fungi and their diversity can drive the breakdown of recalcitrant matter (Setälä & McLean 2004; De Boer *et al*. 2005). In line with this, our results indicated that decomposition and N turnover resulted primarily from reduced fungal diversity. In addition the reduction in size of the organisms inoculated also contributed to the decline in litter decomposition. This highlights potential importance of detrivore soil invertebrate faunal communities and the combined functioning of these organisms with the soil microbial saprophytic community for the cycling of plant available nitrogen (Eisenhauer *et al*. 2010; Eisenhauer 2012).

Similar to earlier studies reporting the presence and abundance of soil microbes ability to reduce leaching of ammonium and phosphorus (Bonkowski & Roy 2005; van der Heijden 2010), we observed soil biodiversity loss reduced the capacity of soils to retain N and P; indicated by total P and NH_4 leached as well as N_2O emissions. Increased loss of N and P via leaching can occur as a result of the loss and simplification of the soil microbial community since many microbes more readily acquire N in the form of ammonium (Merrick & Edwards 1995; Marzluf 1997) and P uptake by plants is the primary function of arbuscular mycorrhizal fungi (Smith & Read 2008). Additionally, gaseous losses of N are a known to be mediated by fungal and bacterial process (Laughlin & Stevens 2002; Ollivier *et al*. 2011), and their suppression may have disrupted microbial N processes leading to increased N_2O production. Leaching of PO_4 and NO_3, is also mediated by plant species composition and productivity (Hooper & Vitousek 1998; Maestre *et al*. 2012). Hence, leaching of these nutrients may also be influenced by changes in plant community composition and productivity and are thus indirect effects of changes in soil biodiversity.

Overall, our study illustrates that biodiversity in soils is a key mechanism behind ecosystem multifunctionality. More specifically soil biodiversity loss may result in the suppression ecosystem processes below their optimum. Currently, however, our study cannot answer which species, taxonomic groups of organisms, or functional guilds of organisms drive each of these functions as well as how these organisms interact to communally support ecosystem multifunctionality. Such aspects of soils will need to be addressed to fully unravel the relationships between soil biodiversity and the mechanisms by which it sustains multiple ecosystem services processes.

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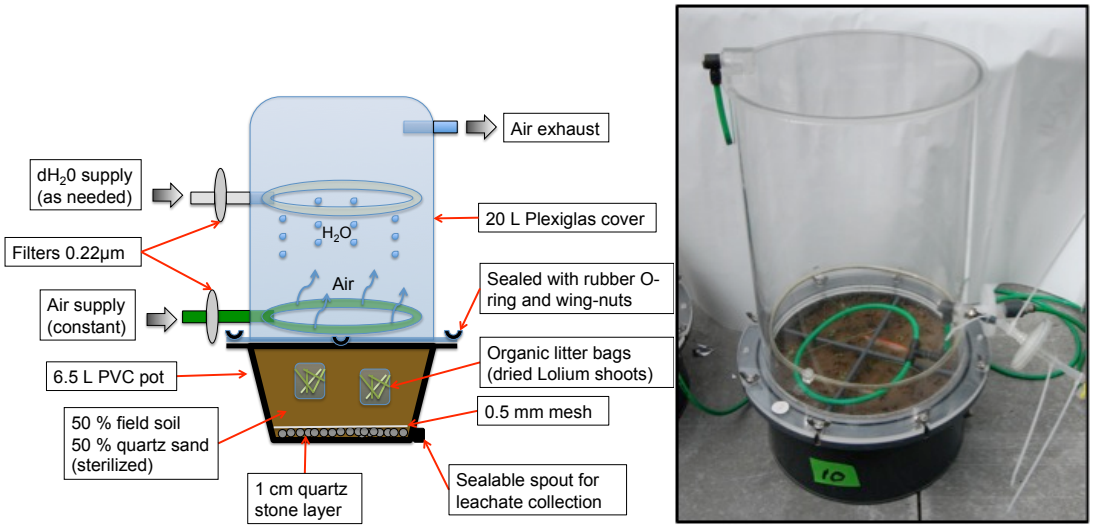
APPENDIX A: Microcosm schematic diagram.



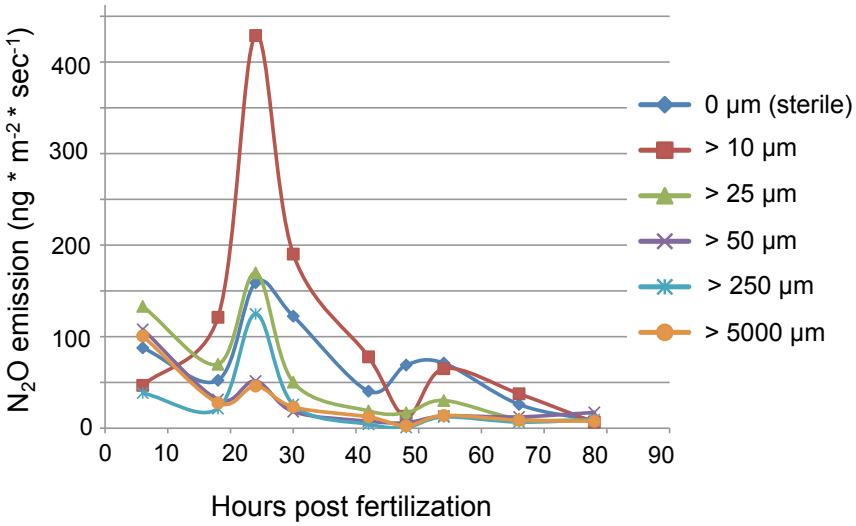
Schematic of microcosm setup (left) and actual microcosm (right).

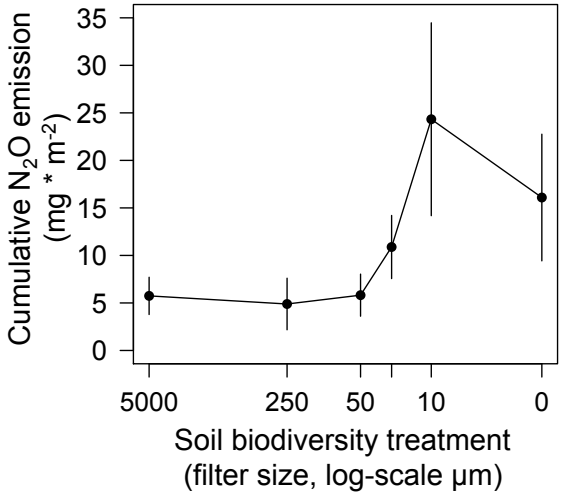
APPENDIX B: Substrate characteristics.

Soil characteristics in microcosms with the five soil inocula treatments from experiment 1. For each variable the overall mean and standard error of the mean (SE) across the five soil inocula treatments are shown. A one-way analysis of variance (ANOVA) with soil inocula treatment as the source of variation was performed to test whether soil characteristics differed among the five different soil inocula treatments. The F-value and the significance level (P) is shown for each ANOVA.

Soil Characteristic	**Mean (SE)**	$F_{4,5}$	**P**
pH	7.56 (0.01)	1.15	0.43
Plant available nutrients			
Water soluble NO_3- and NH_4+	9.54 (0.79) $mg \cdot kg^{-1}$	1.15	0.43
P_2O_5 (CO_2-saturated water extracted)	1.25 (0.01) $mg \cdot kg^{-1}$	2.05	0.23
K_2O (CO_2-saturated water extracted)	0.61 (0.003) $mg \cdot kg^{-1}$	1.00	0.49
Ammonium acetate -extracted mineral nutrients			
Ca	6.51 (0.04) $\times 10^3$ $mg \cdot kg^{-1}$	0.61	0.68
P	21.75 (0.32) $mg \cdot kg^{-1}$	0.41	0.79
K	15.65 (0.11) $mg \cdot kg^{-1}$	0.65	0.65
Mg	4.88 (0.03) $mg \cdot kg^{-1}$	1.15	0.43

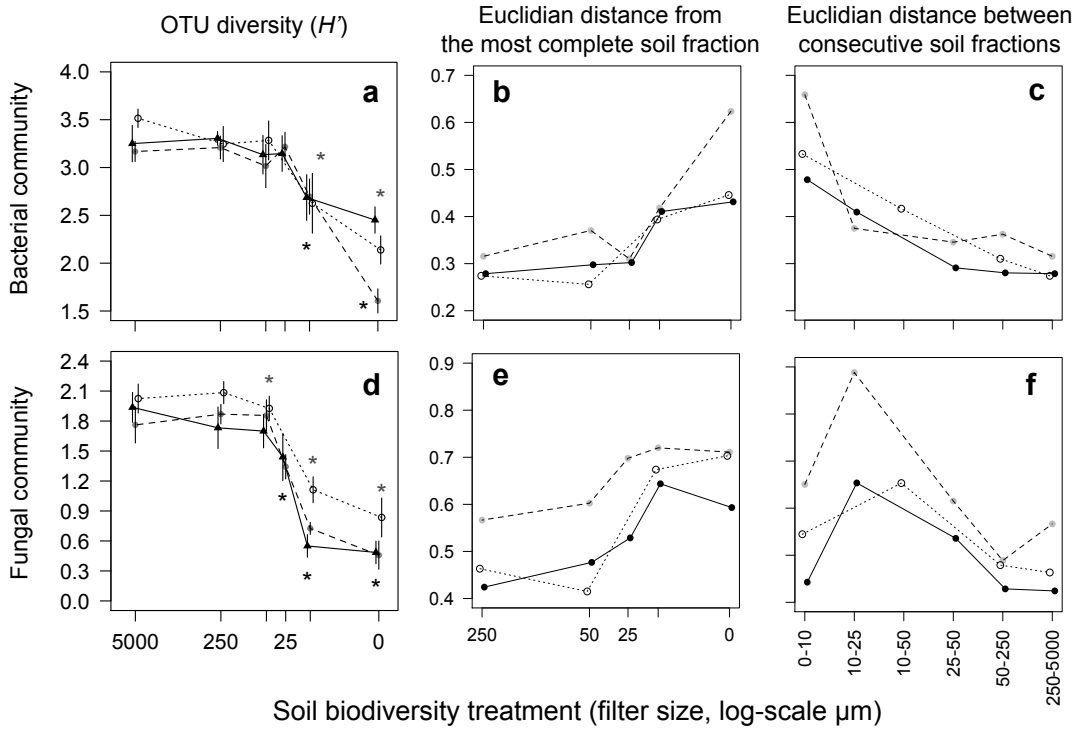
APPENDIX C: N_2O emissions.





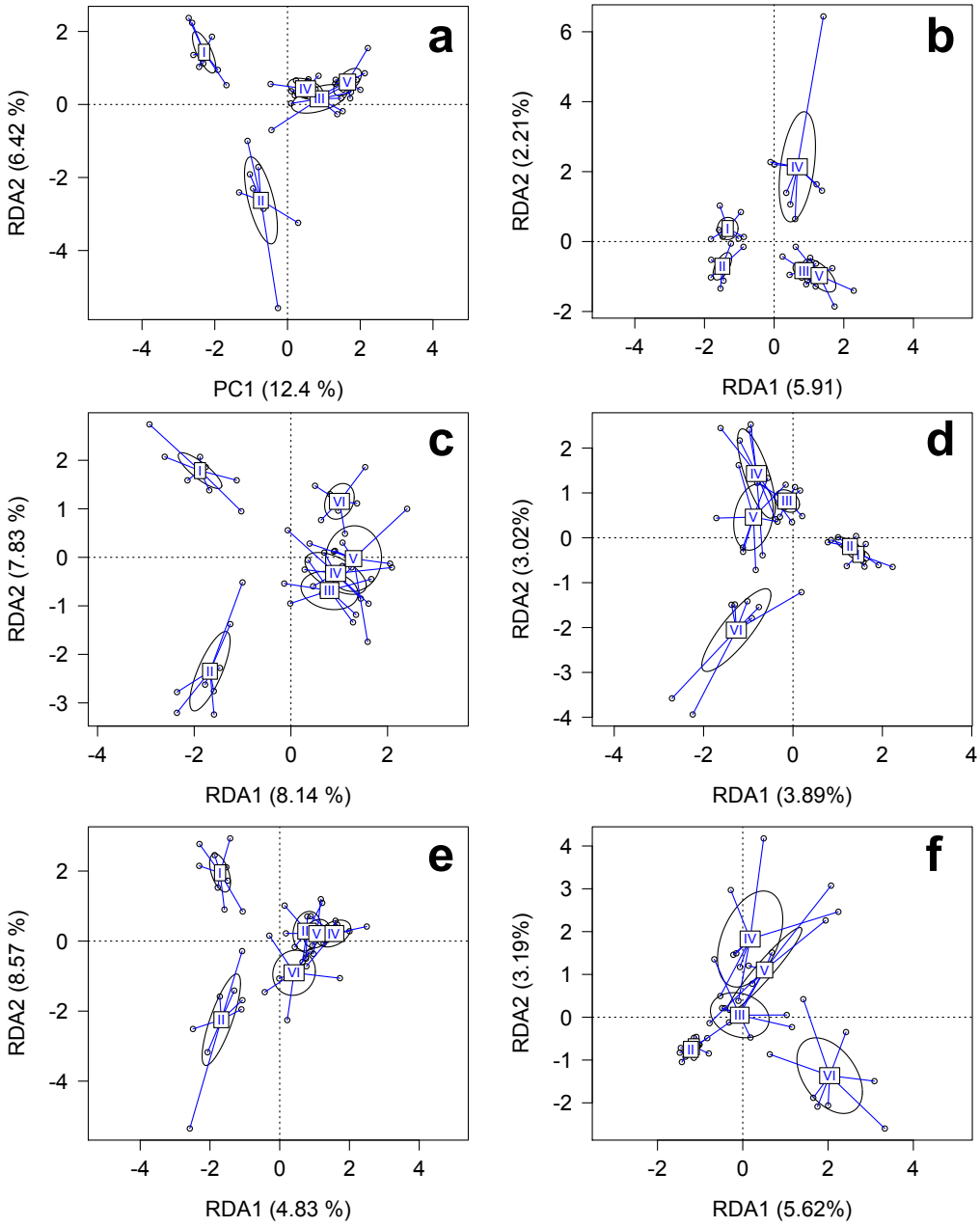
N_2O measurements over time (in hours) after fertilizing and watering the microcosms to field capacity. The cumulative sum of the N_2O measurements for each treatment over the sampling period is assessed as an ecosystem function.

APPENDIX D: Soil microbial OTU community diversity and similarity means.



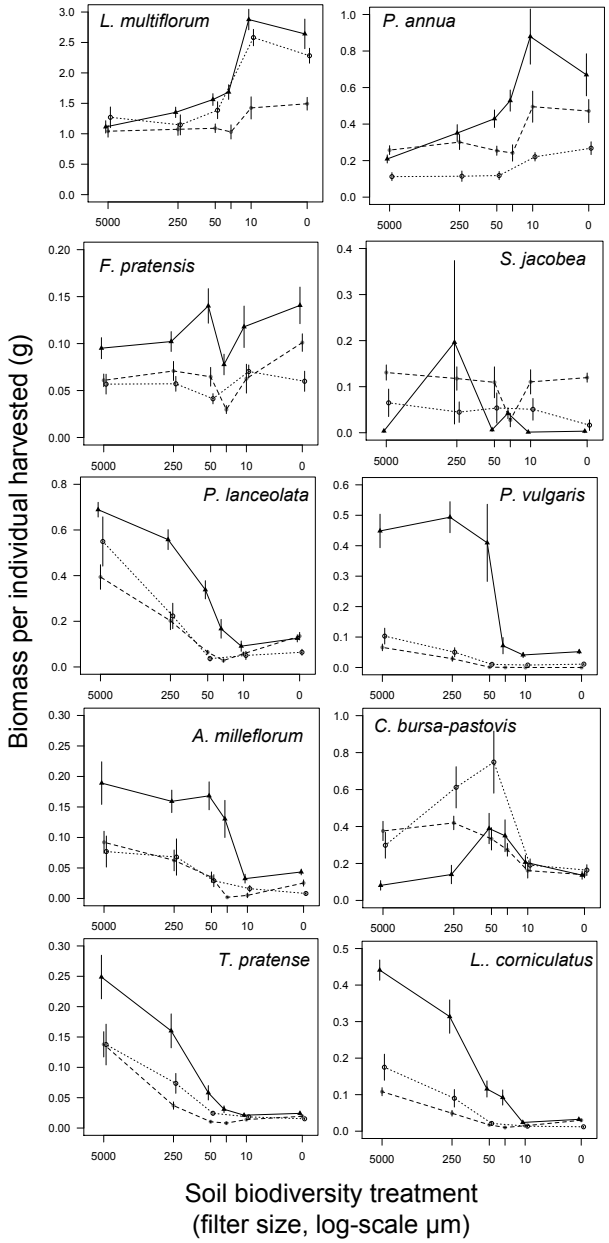
The bacterial (a-c) and fungal (d-f) OTU community characteristics. Panels (a) and (d) show the Shannon diversity index of OTUs, (b) and (e) show the average Euclidean distance from the most complete soil treatment (5000 μm), and (c) and (f) show the average Euclidean distance between consecutive soil biodiversity treatments. Note: the 10-50 μm Euclidean distances are only calculated for 2010 data and 10-25 μm for 2011 data. For all panels, open circles and dotted line = 2010 data, grey circles and dashed line = 2011 data at 12 weeks, and black circles solid line = 2011 data at 24 weeks. Treatments that differed significantly in OTU diversity (panel a and d) from the most complete soil treatment (5000 μm) are indicated by astricts (*) above the means in grey for the 2010 data and in bold below means for the 2011 data (pooled for both harvests).

APPENDIX E: Soil microbial OTU community ordination plots.



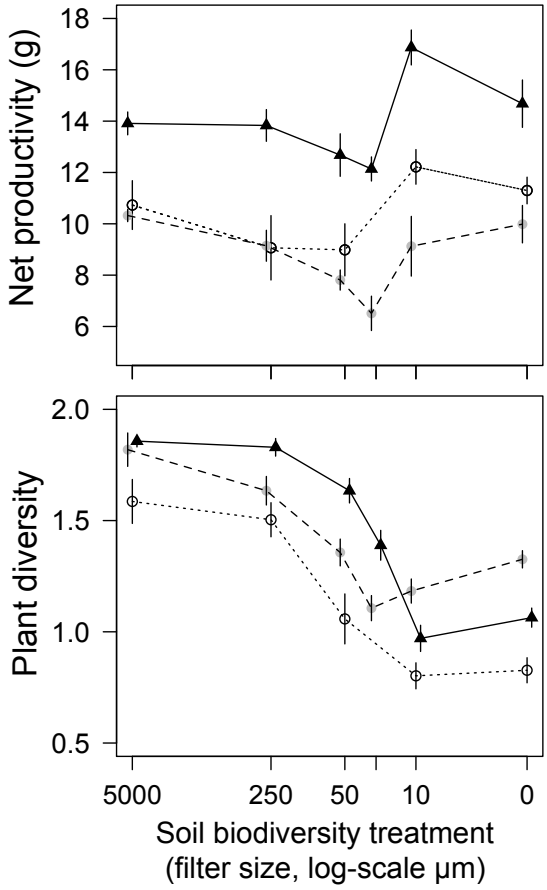
Ordinations of bacterial (left column panels) and fungal communities (right column panels) in different soil biodiversity treatments. The analyses for the 2010 trial are depicted in panels (a) and (b); for the 12 week harvest of the 2011 trial in (c) and (d) and in the final 24 week harvest panels (e) and (f). Constrained redundancy analyses (RDA) were performed to compare the communities. Roman numerals indicate the centroid of the soil community filtration treatments; for (a) and (b): I = sterile inocula, II = < 10 μm, III < 50 μm, IV = < 250 μm and V = 5000 μm; for (c) through (f): I = sterile inocula, II = < 10 μm, III < 25 μm, IV = < 50 μm, V = < 250 μm and VI = 5000 μm.

APPENDIX F: Mean biomass per plant



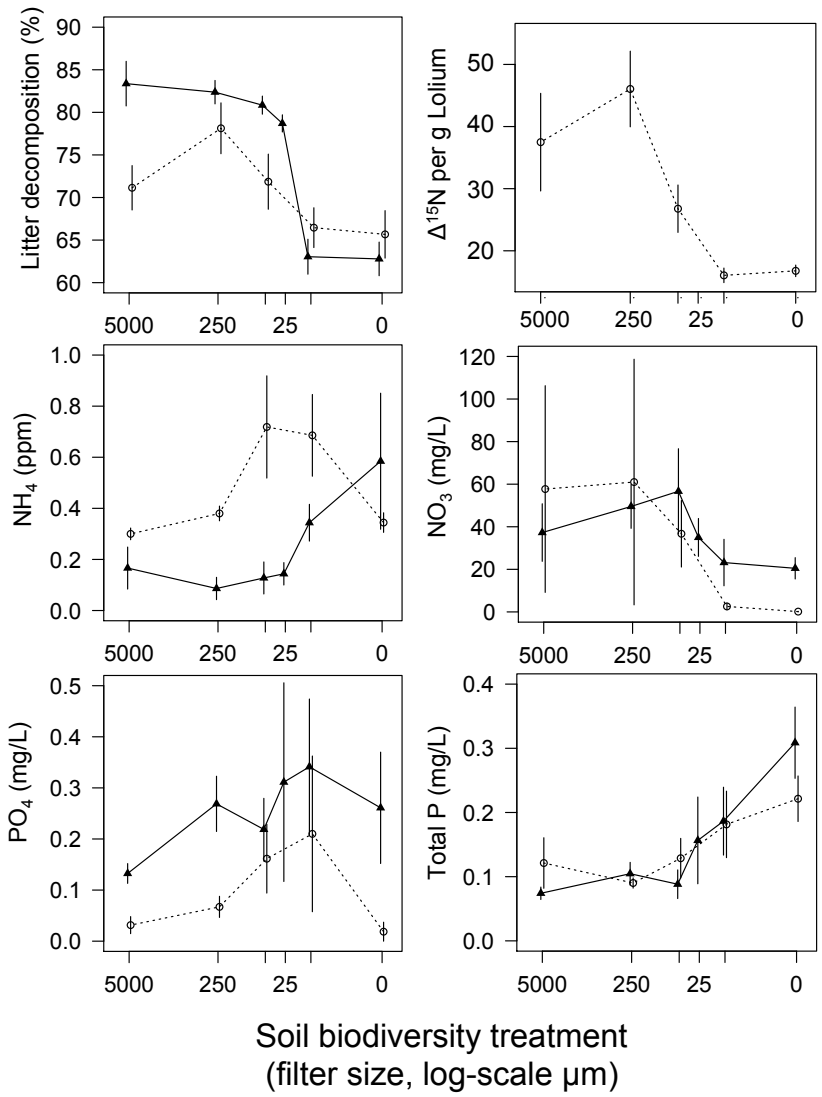
Plant biomass (g) per individual harvested for each of the 10 species. Means and e standard errors of the mean are shown. Lines connecting means are added to visualize trends and points are staggered slightly within each treatment for readability. Dotted lines and open circles are the data collected from the initial trial (2010 after 14 weeks of growth), dashed lines and filled circles are the data collected in the second trial during the first harvest after 12 weeks of growth, and diamonds and solid lines are the data collected in the second trial after 24 weeks of growth.

APPENDIX G: Mean net productivity and plant diversity



Net primary productivity (g) means and standard errors. Lines connecting means are added to visualize trends and points are staggered slightly within each treatment for readability. Dotted lines and open circles are the data collected from the initial trial (2010 after 14 weeks of growth), dashed lines and filled circles are the data collected in the second trial during the first harvest after 12 weeks of growth, and diamonds and solid lines are the data collected in the second trial after 24 weeks of growth.

APPENDIX H: Mean litter decomposition, N turnover, and nutrient concentration leached



Organic litter decomposition, N turnover (^{15}N uptake by *L. multiflorum*) and nutrient losses by leaching in different soil biodiversity treatments; points represent treatment means and bars above and below the mean are standard errors of the mean. Lines connecting means are added to visualize trends and points are staggered slightly within each treatment for readability. Dotted lines and open circles are the data collected from the initial trial (2010 after 14 weeks of growth), and diamonds and solid lines are the data collected in the second trial after 24 weeks of growth. NO_3 data for the 2010 trail is scaled by $x10^2$ for graphical comparisons in trends between trials.

APPENDIX I: Correlation matrix of all ecosystem functions and soil biodiversity characteristics. Filter = filter size (n=140); Fungal = fungal OTU richness (n=140); Bact. = bacterial OTU richness (n=140); M = multifunctionality index (n = 140); NP = net productivity (n = 140); Divers. = plant diversity (n = 140); Total P = total phosphorus leached (n = 85[§]); PO_4 = phosphate leached (n = 85[§]); NO_3 = nitrate leached (n = 85[§]); N_2O = nitrous oxide emitted (n = 50[††]); N turn. = turnover of plant available nitrogen (n = 40[†]); Decom. = litter decomposition (n = 90[§]).

	Fungal	Bact.	M	NP	Divers.	Total P	PO_4	NO_3	NH_4	N_2O	N turn.	Decom.
Filter	0.74	0.54	0.46	-0.09	0.74	0.45	0.11	-0.23	0.26	-0.29	0.51	0.54
Fungal	1.00	0.53	0.35	-0.30	0.67	0.44	0.15	-0.25	0.27	-0.33	0.72	0.58
Bact.		1.00	0.15	-0.14	0.39	0.30	0.07	-0.10	-0.12	-0.02	0.40	0.25
M			*1.00*	*0.47*	*0.70*	*0.69*	*0.61*	*0.17*	*0.61*	*-0.01*	*0.49*	*0.49*
NP				1.00	0.02	-0.14	-0.04	0.40	0.03	0.31	-0.52	-0.44
Divers.					1.00	0.42	0.18	-0.12	0.33	-0.42	0.58	0.57
Total P						1.00	0.69	-0.19	0.46	-0.35	0.20	0.40
PO_4							1.00	-0.06	0.37	-0.16	0.18	0.09
NO_3								1.00	-0.21	0.30	-0.28	-0.27
NH_4									1.00	-0.41	0.26	0.34
N_2O										1.00	NA	-0.31
N turn.											1.00	0.57
Decom.												1.00

Note: § = data comprised from final harvest only of both trials, † = 2010 final harvest data only, †† = 2011 final harvest data only.

Chapter 5

PLANT-SOIL FEEDBACK AS A POTENTIAL EVOLUTIONARY MECHANISM

Wagg C, Boller B, Schneider S, Widmer F & van der Heijden MGA

(*Submitted*)

ABSTRACT

Plant-soil feedback can drive the performance of specific plant species and alter plant community composition. However, its importance for plant evolution has rarely been addressed. We tested whether plant-soil feedback can influence adaptive differentiation in plant populations using parental and daughter populations of diploid and tetraploid *Trifolium pratense* L. cv. associating with soil biota from their native and non- native sites. When inoculated with native soil, diploid and tetraploid populations demonstrated specific root-associated microbial communities. Tetraploid plants benefited the most while diploid plants benefited the least when associating with their native soil biota. Moreover, generational differences were population specific. Biomass of tetraploid daughter population increased 42% relative to the parental population when inoculated with soil communities conditioned by their parental population. Conversely, diploid daughter and parental populations responded similarly to their native soil communities. The results suggest that plant-soil biota feedback effects can influence divergent adaptation between intraspecific plant populations.

INTRODUCTION

Interactions between plants and soil are tightly interlinked (van der Heijden *et al.*, 2008; Bardgett & Wardle, 2010). Plants influence belowground soil communities, such as symbiotic mycorrhizal fungi or pathogenic microbes, that 'feedback' with increased or decreased beneficial effects on the succeeding plant (van der Putten *et al.,* 1993; Bever *et al.*, 1997; Bever, 2003). This mechanism has been known to drive ecological processes in plant communities, such as plant community succession (van der Putten *et al.*, 1993; Kardol *et al.*, 2007), plant species invasions (Callaway *et al.*, 2004; Reinhart & Callaway, 2006), maintenance of plant diversity (Petermann *et al.*, 2008), and drive plant species rarity (Klironomos, 2002). However, less discussed is the fact that this bidirectional relationship may operate on intraspecific heritable characteristics and thus incur evolutionary processes (van Breeman & Finzi, 1998).

For instance, evolution in plants via adaptive divergence arises from the interplay between the local ecosystem and heritable plant characteristics (Rieseberg & Willis, 2007); such as the interaction between plants and their associated belowground communities. Soil microbial communities influence the performance of their hosts via mutualistic and antagonistic interactions and thus may influence the selective pressure on plants for improved mutualistic or reduced antagonistic interactions (van Breeman & Finzi, 1998; Lau & Lennon, 2011; Hoeksema, 2010). Although empirical evidence for this process is largely missing, different genotypes and cultivars have been shown to influence their associated soil microbial communities and their functioning (Easton *et al.*, 2001; Schweitzer *et al.*, 2004; Kiers *et al.*, 2007; Silfver *et al.*, 2007; Wagg *et al.*, 2011a). This provides the foundation for genotypic differences among plant populations to differentially drive plant-soil feedback effects that influence progeny performance and adaptive characteristics. Such an evolutionary interaction between plants and soil organisms has been thought to be one of the mechanisms for the success of invasive plant populations (Seifert *et al.*, 2009).

The ecological differentiation in plant populations requires genetic variation between populations that interacts with the local environment. Such variability in plants can result from breeding and genome duplication events (Reiseberg & Willis, 2007; Van de Peer *et al.*, 2009; Wood *et al.*, 2009). Polyploidy, or genome duplication, occurs frequently in flowering plants (Otto & Whitton, 2000) and can alter the adaptive potential of plants to environmental characteristics (Levin, 1983; Ramsey & Schemske, 2002; Flagel & Wendel, 2009). This has intrigued many

evolutionary ecologists, as it would suggest differences in ploidy can alter the environmental selection that drives adaptation through associated genetic diversification, gene dosage, and morphological effects (Reiseberg & Willis, 2007; Flagel & Wendel, 2009). Such genetic and functional differences between plant populations differing in ploidy provide an excellent model for exploring evolutionary mechanisms behind the ecological differentiation in plants.

Here we test the hypothesis that genetic variation between populations, reflected in pedigree and ploidy differences, influences progeny performance via plant-soil feedback. This infers that not only should root associated communities be plant population specific (eg. Freckleton & Lewis, 2006), but also that plant performance should also be influenced most by its native soil community than non-native communities. Moreover, in view of the rapid genetic and adaptive changes that can occur and the increased genetic diversity that polypoloidy infers (Leitch & Leitch, 2008; Flagel & Wendel, 2009) we hypothesize that tetraploid progeny plants (4x) are better adapted to their soil biotic communities than a conspecific diploid (2x) population.

In order to test this hypothesis we used a bioassay experiment to test plant growth responses to native and non-native soil communities of two *Trifolium pratense* L. (red clover) *cv.* allogamous populations. These two populations differ in ploidy and pedigree, both heritable traits influencing plant characteristics. We utilized these two populations as a model plant system for assessing soil feedback effects on plant responses between generations. We chose *T. pratense* for its broad use and value in agriculture (Boller *et al.*, 2010) and since *Trifolium* sp. are known to depend heavily on soil microbial symbiotic interactions with rhizobia and AMF for the uptake of the majority of their acquired N and P (Boller & Nösberger, 1987; Feng *et al.*, 2003). We also chose these two populations for their differences in ploidy since adaptive ability in plants can be influenced by ploidy. Although the two populations also differ in pedigree, we refer to these two populations as diploid (2x) and tetraploid (4x) for simplicity.

METHODS

Seed, soil and inocula

Seeds from two different populations of *T.pratense* were used in this study: one 4x population and one 2x population. Tetraploid *T. pratense* seeds were created by colchicine induced genome duplication from the variety Formica (Boller, 1996), followed by 5 generations of propagation, such that the 'parental' seed population is the 6th generation post genome duplication and 2nd generation post selection of individuals (2nd synthetic population; *Syn-2*). Diploid *T. pratense* seed originated from intercrossing the diploid varieties Milvus (Boller & Nüesch, 1995) and Corvus (Boller, 2000). Seeds of the 'parental' diploid hybrid variety were collected from the 4th generation post intercrossing and 2nd generation post selection of individuals (*Syn-2*). Both parental seed populations were sown into well-isolated fields in early summer 2008 as monocultures at different sites. The 4x population was located near the Reckenholz site of the research station ART (47° 42' 55'' N, 008° 54' 02'' E) and the 2x population 30 km away near the Tänikon site of the research station ART (047° 47' 54'' N, 008° 90' 96'' E). Plots of *Lolium* sp. monocultures were also grown at each of the two sites during this time. Progeny seeds (*Syn-3*, here on referred to as 'daughter' population) were harvested in bulk from the two clover populations in August 2010. Seed mass did not vary between daughter and parental populations (sampling 100 seeds per population) in both the 2x population (daughter = 2.11 mg, SE=0.29 and parental = 2.10 mg, SE=0.35; P=0.98) and 4x populations (daughter = 2.80 mg, SE=0.39 and parental = 2.87 mg, SE=0.37; P=0.18).

In order to study plant-soil feedback, soil inoculum was collected from below *T. pratense* plants using soil cores (2.5 cm diameter, 10-12 cm in depth). In addition soil cores were also taken from plots in which *Lolium* sp. monocultures were grown as a control for site-specific inocula effects resulting in a total of four plots in which soils were sampled. In each of the four plots soil cores were taken approximately every 0.5 m along 4 transects across each plot, until roughly 5 kg of soil was collected. The soil cores were pooled and homogenized for each plot by sieving through a 5 mm mesh. Half of each of the four soil inocula were sterilized by autoclaving for 60 min at 121 °C.

The experiment was performed in 1L pots filled with 800 g of sterilized substrate (a mix, by weight, of 50-50 quartz sand - field soil from a local grassland (see Wagg *et al.* 2011b), that was sterilized by autoclaving for 60 min at 121 °C). Each pot received 90 g (approximately 7.5 % of total substrate by weight) of either

living or sterilized soil inocula of one of the four plots resulting in 8 inocula treatments: 2 soil origins (2x or 4x site) × 2 soil histories (clover or lolium-conditioned) × 2 soil treatments (sterilized or unsterilized). The inocula were mixed throughout pots. Pots were subsequently toped up to 1100 g with additional sterilized sand-soil substrate. Two additional pots of each of the 8 inocula treatments were created and the inoculated substrate characteristics analyzed.

There were four seed population treatments: 2x seeds from the 2x sites (daughter population) and the 4x seeds from the 4x site (daughter population) as well as the original 2x and 4x seed populations initially sown into the two fields 3 growing seasons prior (the parental population). Seeds were surface sterilized by slow agitation in 50 % household bleach (roughly 2.5% sodium hypochlorite) with a few drops of Tween 20 for 5 min. Seeds were rinsed with dH_2O and allowed to germinate on 1% water agar for 3 days. Subsequently, in each pot, six *T. pratense* seedlings of a single population (4x or 2x) and generation (parental or daughter) were transplanted into each pot. Seedlings that did not survive the first week post planting were replaced.

Despite the low percentage of inocula in the substrate, soil characteristics varied due to sterilization of the inocula as well as its origin (see Appendix A for details). Although these differences were detected it likely held little bearing on plant performance as not only is *T. pratense* known to be more sensitive to the presence and diversity of root associated symbionts than substrate characteristics (see Wagg *et al*., 2011c) and grew poorly in treatments with sterilized inocula (see results). Furthermore, these differences are reflected and accounted for by the overall inocula 'origin' effect in all analyses (see below).

Experimental design

Each of the 8 inocula treatments × 2 *T. pratense* daughter population combinations were replicated 10 times in a complete factorial design for a total of 160 pots. In order to test for differences between parental and daughter plants in their performance with their native soil communities, parental 2x plants were grown in pots with either the sterilized or living soil originating from under the 2x plants and parental 4x plants were grown in pots with either the sterilized or living soil originating from under the 4x plants with 10 replicates of each for an additional 40 pots (200 pots total). Pots were randomly distributed within the greenhouse and grown under natural light conditions subsidized by 400-W high-pressure sodium lamps to maintain a light

level above 300 W/m^2 so that growth conditions could be maintained as 16 h / 25 °C days and 8 h / 16 °C nights. Soil moisture was maintained between 20-30 % by weight. Plants were harvested 14 weeks post initial planting and a census of mortality and the number of flower heads per pot recorded. Leaves, stems and flower heads were separated and dried at 60 °C and their biomass recorded. Roots were washed clean of soil and frozen at -20 °C until they could be processed further.

Processing of roots

Roots were thawed, fresh weight recorded and cut into small pieces, 1–2 cm in length in cold water. A random sample of 0.5 g of fresh root was selected and fixed in 50 % ethanol for scoring AMF root colonization. These roots were then cleared and stained with 0.05 % (w/v) trypan blue in lactoglycerol (see Brundrett *et al*., 1994). Stained roots were mounted on slides and scored for presence of AMF structures (arbuscules, vesicles, hyphae) following the transect method outlined in McGonigle *et al*., (1990) for 100 intersects. Colonization by non-AMF structures was also noted (such as dark septate hyphae, microsclerotia, as well as fine or hyaline hyphae, vesicles and spores). Pots receiving sterilized inocula were also checked for nodulation and AMF colonization. Plants in 9 pots receiving sterilized inocula (see Appendix B) exhibited AMF colonization and or root nodulation and were excluded from the data set.

Three root systems were randomly selected per pot and 10 root nodules were removed and lyophilized for a total of 30 root nodules per pot. These were then weighed and used for molecular assessment to ensure DNA extracted from roots included DNA of nodule forming microbes. The remaining root system was lyophilized and 20 mg of lyophilized roots was added to the lypholized nodules (approximately 4 mg) for DNA extraction using the Qiagen DNeasy plant mini kit following the manufacturer recommendations for the purification of total DNA from plant tissue (Qiagen Sciences, Germantown, Maryland, USA). Since roots of plants grown in substrate inoculated with sterilized soil were insufficient in size for sampling for both AMF and root biomass assessment, half of the replicates were randomly selected to be either lyophilized, dry mass recorded or fixed in 50 % ethanol and used for assessment of AMF root colonization. Molecular analyses were performed with roots in treatments inoculated with sterile soil providing sufficient root mass, post removal of roots for microscopy.

Molecular analysis of root associated microbial communities

Root associated bacterial community profiles were assessed using ribosomal internal spacer analysis (RISA). The RISA was done using PCR reactions with the extracted DNA diluted to 10 ng μl^{-1} previously quantified using PicoGreen® (Molecular Probes, Eugene, OR) on a Cary Eclipse Fluorescence Spectrophotometer. The RISA primers used for root associated bacterial communities were bRISArev and bRISAfor provided in (Hartmann *et al*., 2005) and using PCR reagent concentrations and cycling conditions provided in Hartmann *et al*., (2005).

In order to assess the variation in the size of the amplified fragments from bacterial PCR reaction, 2 μl of the PCR product was mixed with 12 μl HiDi-Formamid and 0.2 μl MapMarker® 1000 (BioVentures, Murfreesboro, TN) as the size standard and subject to fragment analysis using an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Run conditions were set to 7 kV and 60 °C with a run time of 3000 s. The relative migration units were then assessed using GenMarker 1.5 genotyping software (SoftGenetics LLC, State College, PA) and in each profile unambiguous fragment peaks were used as operational taxonomic units (OTU). Peak intensities of the OTUs were scored as relative florescence units with a threshold value of 50 units. Bacterial OTU peak intensities were converted to proportions of the total peak intensity sum in each sample in order to reduce the amount of variation due to variation in PCR amplification among samples following Hartmann *et al*., (2005).

Fungal associated communities were also assessed with RISA using primers targeting the ITS1-5.8S-ITS2 region (see Sequerra *et al*., 1997; Ranjard *et al*., 2001) and following the PCR reagent concentrations and cycling conditions outlined in Schneider *et al*., (2010). These primers do not amplify AMF and only 7 OTU peaks were detected in total and an average of 2 fungal OTUs per treatment group were amplified from the root extracted DNA. In view of these limitations, this data provided little variation for meaningful interpretations and are not presented.

Data analysis

Since not all plants survived until harvest (see Appendix B) resulting in differences in the number of plants per pot, all plant biomass measures were assessed as the biomass per surviving plant. The response in aboveground plant productivity to the inoculated soil communities was assessed as the $\log_e$ ratio of the living (l) to sterilized (s) aboveground biomass such that response = $log_e(l_i / s_i)$,

where l_i is the observed biomass when inoculated with living inocula of treatment combination *i* (inocula origin, history and plant ploidy and generation combination) and s_i is the mean biomass of same plant population inoculated with sterilized soil of the same history and origin. This measure reflects the benefit to plant growth by associating with field soil borne biota including symbiotic root associated microbes (AMF and rhizobia) relative to associating with greenhouse borne microbes in the absence of root associated microbial symbionts. All statistics and calculations were carried out using the software R version 2.13.0 (The R Foundation for Statistical Computing 2011). There was ≤10 % mortality in all treatments except the 2x daughter plants inoculated with sterile soil (25-35 % mortality; see Appendix B). Therefore mortality was added as a covariate in all ANOVAs to counteract plant density dependant effects on plant performance. Bacterial OTU data were square root transformed prior to ANOVA to improve homoscedasticity.

Plant and root associated bacterial community and fungal colonization characteristics of the daughter plants inoculated with living soil were assessed using ploidial level, inocula origin and history in a three-way ANOVAs. Contrasts were used to determine whether characteristics differed between native and non-native inoculated soils and additionally to test whether differences occurred between ploidial levels when inoculated with the same soil. To test whether daughter plants differed from their parental plants when associating with their native soil communities, two-way ANOVAs with generation and ploidy and their interaction as sources of variation were used and differences between the parental and daughter lines in both the 2x and 4x plants were determined by contrasts.

OTU community analyses were carried out using functions found within the package 'vegan' (version 1.17.11 for R; see Oksanen *et al.*, 2011). Prior to multivariate anylses, bacterial and fungal OTU data matrices were standardied by z-score transformations of each OTU column (mean = 0, SD = 1) and each row in the data set normalized; such that the sum of squares of each row = 1. Bacterial community structure was assessed in a similar manner as the plant characteristics. Three-way multivariate ANOVAs were carried out on Euclidean distance matricies, calculated using 'vegdist' function on bacterial OTU data, to determine whether detected communities differed depending on inocula origin, history and the ploidy level of the daughter lines as well as their interactions among factors using the function 'adonis' with 200 permuitations to determine *pseudo*-F values for the level significance. Differences in bacterial community structure were further assessed and

visualized by redundancy analysis (RDA) using the function 'rda' (see Bocard *et al.*, 2011) on the bacterial OTU data constrained by the same factorial designs as for assessing Euclidean distances. The differences between bacterial OTU community structures of parental and daughter plants were compared using Euclidean distances in a two-way multivariate ANOVAs to assess the variation using function 'adonis' with ploidy-level and generation as well as their interaction as sources of variation, followed by redundancy analysis.

Plant population – bacterial OTU community interactions were further visualized in a bipartite network and interaction matrices using absence / presence data for the bacterial OTU community using package 'bipartite' for R (see Dormann *et al.*, 2008). OTUs must have occurred in at least 3 replicates of one plant population to simplify the networks.

RESULTS

Effects of inocula origin, history and plant ploidy

Aboveground biomass varied significantly among inocula origin, history and plant ploidy combinations when inoculated with living soil (Table 1a). Both 4x and 2x daughter plants produced greater aboveground biomass when inoculated with their native soil than when inoculated with non-native soil (Fig. 1a, P=0.007 and P=0.005, respectively). However, 2x daughter plants inoculated with sterilized soil also produced a similar trend (see Fig. 1b; Table 1b), suggesting the effect reflects benefits from edaphic soil characteristics rather than its natural biotic community. Additionally, plants inoculated with their native living soil community demonstrated greater productivity than the reciprocal cytotype when inoculated with the same inocula, but only significantly so when inoculated with 4x native soil (Fig. 1a). Importantly, inoculation with lolium-conditioned soil did not reflect a similar origin specific effect and neither was this trend reflected in the aboveground productivity of plants receiving sterile inocula (Fig. 1b), thus indicating plant performance with native soil is a result of interactions with the soil biota inoculated. The response in aboveground productivity (Fig. 1a and b) revealed the differences in soil feedback effects between 2x and 4x plants (Fig 1c). As observed in aboveground biomass, the response also varied significantly among the combinations of inocula origin, history and the ploidal level of the plants (Table 1c). The 4x daughter plants showed strong positive soil feedback effects by benefiting the most when inoculated with their native community than all other inocula (Fig. 1c). Contrary to this, the 2x daughter plants

benefited the least from inoculation with their native soil community than all other inocula, reflecting negative feedback. The 2x plants benefited most from the lolium-conditioned soil, reflecting heterospecific positive feedback.

Belowground biomass was similar in trend to the aboveground biomass (Fig. 1d). However, 4x daughter plants did not differ among inocula treatments ('native' versus 'non-native contrast P=0.18), while 2x daughter plants produced less root biomass when inoculated with its native soil (native versus non-native contrast P=0.02). This difference between above and belowground biomass in native versus non-native effects reflects the ability of plants to allocate biomass to aboveground organs when associating with the different soil communities. Tetraploid plants were able to allocate 68 % of total biomass to aboveground organs with their native soil community compared to 64 % with non-native inocula, while 2x plants consistently allocated roughly 65 % of their biomass to aboveground productivity across all inocula treatments.

Flowering was rare in 4x plants while 2x plants consistently flowered by the end of the growth period (see Appendix B). This is likely a result of 4x plants not receiving the required time to flower, as polyploids are generally slower to flower (eg. Husbund 2000). However, 2x flower head mass correlated well with aboveground biomass (spearman's rho = 0.62, P<0.001) and thus allocation of biomass to reproductive organs is reflected in aboveground productivity.

Colonization by AMF structures and root nodules occurred in every pot that was inoculated with living soil. AMF colonization did not depend on plant ploidy, but only varied depending on the combination of the inocula origin and the history of the inocula (Table 1e, Fig. 1e). Diploid plants had the greatest root length colonization when inoculated with their native soil than other inocula (native versus non-native contrast P<0.001). However, AMF colonization was generally greatest in all plants with the native 2x inocula illustrating the effect of origin and history interaction. No native versus non-native effect of AMF colonization was found in 4x plants (P=0.41). Similar to AMF, colonization of daughter plants by non-AMF structures only differed among the origin and history of the inocula ($F_{1,75}$=8.20, P=0.005 and $F_{1,75}$=10.1, P=0.002, respectively). Overall clover-conditioned inocula resulted in greater incidence of non-AMF structures (24%, SE=1.7) than lolium-conditioned inocula (18% SE=1.2) and inocula from the 2x site was greater (24%, SE=1.7) than the 4x site (19%, SE=1.3).

Table 1. ANOVA results for the partitioning of the variation in plant productivity and root associated microbial characteristics among inocula Origin (2x site and 4x site), History (clover or lolium conditioned soil) and the Ploidy level (2x or 4x clover population) of the daughter plants grown with living soil inocula (living). Response in plant aboveground biomass to soil communities is the log-scale ratio of living to sterilized aboveground biomass. The same sources of variation were used for assessing the aboveground biomass of plants inoculated with sterile soil (str).

		(a) Aboveground (living)		(b) Aboveground (str)		(c) Response	
Source of variation	df	MS $\times 10^{-2}$	F	MS	F	MS	F
Origin	1	49.5	49.1 ***	24.6	66.7 ***	21.4	16.2 $\times 10^{2}$ ***
History	1	0.97	0.96	< 0.01	< 0.01	0.40	30.2 ***
Ploidy	1	25.6	25.4 ***	16.4	44.4 ***	22.8	17.2 $\times 10^{2}$ ***
Origin:History	1	2.19	2.17	1.60	4.35 *	1.54	116 ***
Origin:Ploidy	1	19.8	19.7 ***	4.80	13.0 ***	8.93	673 ***
History:Ploidy	1	13.0	12.9 ***	8.06	21.9 ***	12.4	934 ***
Origin:History:Ploidy	1	56.2	55.7 ***	1.40	3.81	0.34	25.6 ***
Residuals	71	1.01		0.39 (df=64)		0.01	

		(d) Belowground		(e) AMF colonization		(f) Bacterial OTU richness	
Source of variation	df	MS $\times 10^{-2}$	F	MS $\times 10^{2}$	F	MS $\times 10^{-1}$	F
Origin	1	34.1	39.9 ***	0.19	0.67	22.8	1.57
History	1	5.37	6.27 *	29.5	29.0 ***	23.9	1.65
Ploidy	1	10.3	12.1 ***	0.52	0.51	1.24	0.85
Origin:History	1	1.07	1.25	55.9	54.9 ***	2.36	0.16
Origin:Ploidy	1	3.44	4.02 *	0.30	0.29	95.2	6.57 *
History:Ploidy	1	1.58	1.84	0.41	0.40	8.20	0.57
Origin:History:Ploidy	1	5.85	6.83 *	0.13	0.13	44.5	3.07
Residuals	71	0.86		1.02		14.5	

Note: df= degrees of freedom, MS = mean squares, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

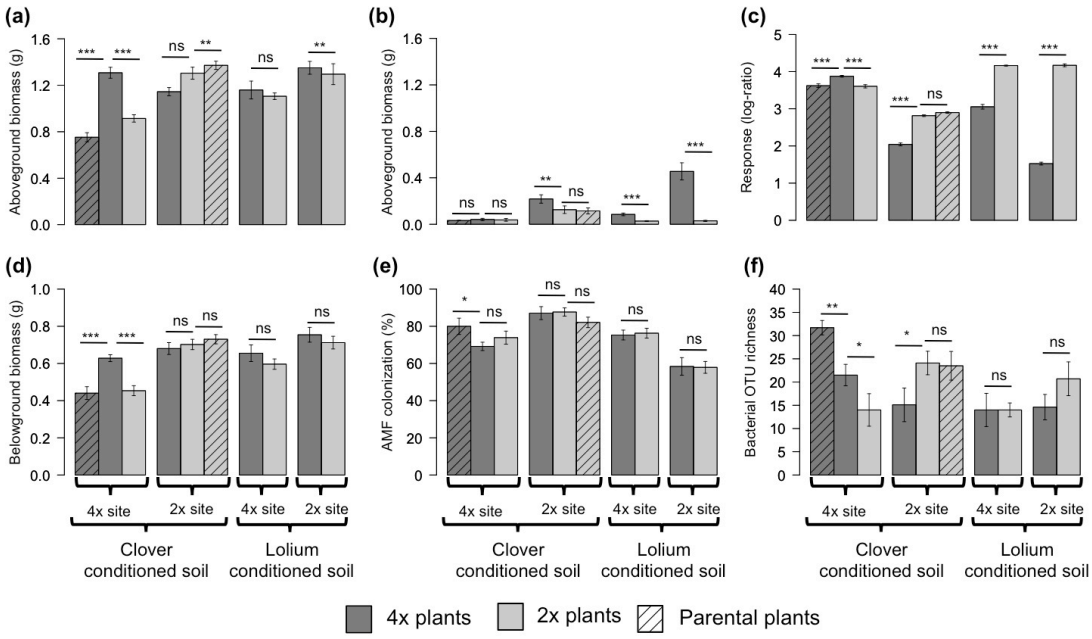


Figure 1. Means and standard errors of plant and root associated microbial community characteristics in treatments with soil inocula from two sites (4x or 2x site), previously conditioned by clover or lolium and using different seed populations (4x or 2x ploidy level populations and parental or daughter generations). Biomass measures are per number of individual plants surviving at harvest. Response to soil biota (c) reflects differences in aboveground biomass of plants inoculated with living soil (a) proportional to aboveground productivity the same sterile soil (b). Bars over means indicate the specific pair-wise contrasts made and their significance after accounting for plant mortality are indicated by ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Differences between parental and progeny characteristics

Aboveground biomass in daughter and parental plants differed depending on whether the plants were 2x or 4x (ploidy × generation interaction term: $F_{1, 35} = 121$, $P < 0.001$; Fig. 1a). Tetraploid daughter plants produced 42 % more aboveground biomass than the parental plants, while the 2x daughter plants produced less aboveground biomass than their parents and the discrepancy between daughter and parental 2x plants was much smaller (5 % less) than what was observed in the 4x plants (Fig. 1a). The response in aboveground productivity also showed population specific differences between parental and daughter plants. Tetraploid daughter plants had a greater positive response than their parental plants (Fig. 1c), while 2x parental and daughter plants showed no difference (ploidy × generation interaction term: $F_{1, 35} = 27.5$, $P < 0.001$).

Root biomass also differed between parental and daughter plants depending on their ploidy ($F_{1, 35} = 16.4$, $P < 0.001$), where 2x parental and daughter plants produced similar root mass while 4x daughter plants produced a significantly greater

root mass than their parental plants (Fig. 1d). No difference between generations in 2x flower head mass occurred (P=0.99, Appendix B).

Colonization of roots by AMF was similar between parental and daughter 2x plants but was less in 4x daughter plants than the 4x parental plants (Fig. 1e, ploidy × generation $F_{1, 35} = 6.02$, $P = 0.02$). Additionally, this same trend was also observed for the colonization of non-AMF structures in roots (ploidy × generation $F_{1, 35} = 5.03$, $P = 0.03$), where 4x parental plants had higher incidence of colonization by non-AMF structures (36%, SE=3.3) than their daughter plants (24%, SE=3.1), but no difference occurred between the 2x daughter and parental populations (27%, SE=3.8 and 24%, SE=3.3, respectively).

Root associated bacterial community

Bacterial OTU richness varied among inocula origin and plant ploidy combinations (Table 1f). Bacterial OTU richness associated with 4x and 2x daughter plant roots was greatest when inoculated their native soil (native versus non-native contrast p=0.03 and P=0.02, respectively; Fig. 1f). Bacterial OTU richness was also greatest in the daughter plants native to the inoculated clover conditioned soil (Fig. 1f). This trend was not observed when plants were inoculated with lolium-conditioned soil. The 4x daughter plants associated with a lower bacterial OTU richness than their parental plants, while 2x parental and daughter plants did not differ (Fig. 1f; ploidy × generation: $F_{1, 35} = 4.21$, $P < 0.05$).

The Euclidian distance among bacterial OTU communities varied among inocula origin, history and plant ploidy combinations (three-way interaction: *pseudo*-$F_{1,72}=1.74$, P=0.005). Overall, bacterial communities of 2x and 4x sites varied from each other (Fig. 2a). Moreover 4x and 2x clover populations differed from each other when inoculated with clover-condition soil from either site (Fig. 2a). Conversely, bacterial OTU communities were similar between 4x and 2x daughter plants when inoculated with lolium-conditioned soil from either site (Fig. 2a). Interestingly, bacterial community structure on roots of 2x populations did not differ between parent and daughter while bacterial communities of 4x populations differed between parent and daughter (ploidy × generation interaction *pseudo*-$F_{1, 36} = 2.41$, $P = 0.01$; Fig. 2b).

The occurrence of individual bacterial OTUs associated with roots of the different clover populations revealed non-native population (eg. 4x plants inoculated with 2x soil and vice versa) interacted the least with the bacterial community detected (Fig. 3a and b). This indicates the 2x and 4x populations have the strongest

interaction with bacteria from sites from which they originated. The 4x parent population associated with the most bacterial OTUs when inoculated with its native soil, while both 2x parent and daughter population shared the highest number of bacterial OTU associations when inoculated with their native soil (Fig. 3; also see Appendix C). This trend was not reflected in the bacterial OTU associations when inoculated with lolium-conditioned soil, with the possible exception of plants inoculated with lolium-conditioned 2x plants (Fig. 3c and d; also see Appendix C). Significant differences in the number of OTU associations among plant populations and inocula are reflected in the OTU richness (see Fig. 1f). This would suggest the bacterial OTU community to be more 'specialized' in associating with each of the different ploidy populations and interact differently depending on the generation of the 4x plants.

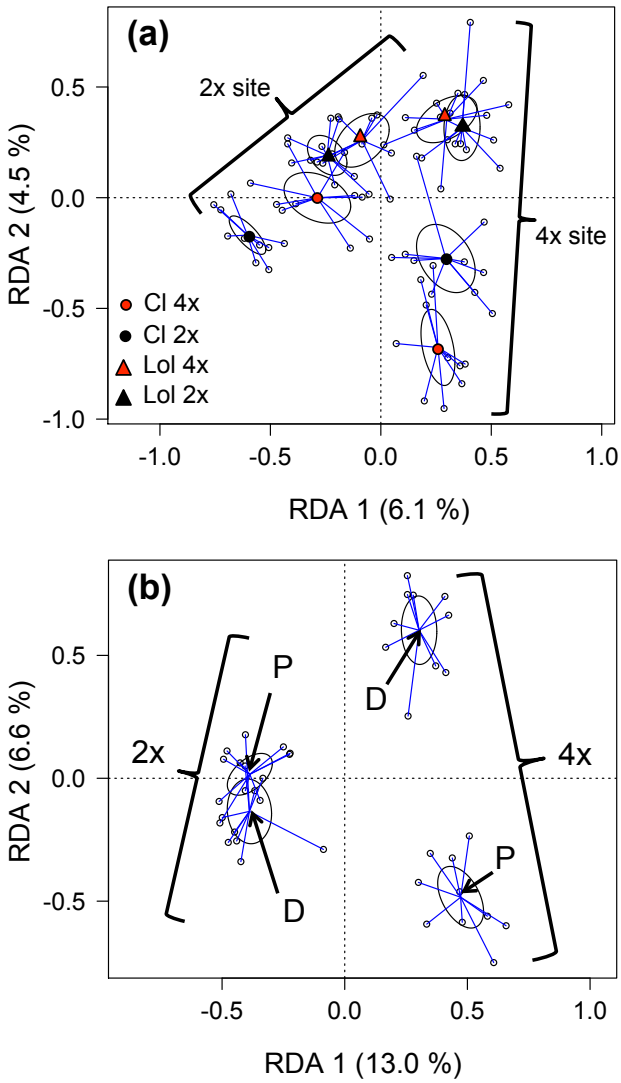


Figure 2. Ordination axes 1 and 2 of the RDA on the bacterial OTU community data associated with (a) the 4 different clover populations (all daughter populations) grown in pots inoculated with clover- or lolium-conditioned soils (Cl and Lol respectively) from two sites (2x and 4x) and (b) the two parental and daughter plants of the two ploidy populations (2x and 4x). Blue lines illustrate distances between each replicate (open circle) and the centroid of each of the groupings by (a) inocula origin (4x or 2x site), soil history (clover or lolium conditioned) and the ploidy level of daughter plants and (b) ploidy level (2x of 4x) and generation (daughter = D, parental = P). A black ellipse indicates the 95 % confidence range around each of the centroids. In panel (a), centroids marked with circles indicate daughter plants inoculated with clover condition soil (Cl), while triangles indicate plants inoculated with lolium conditions soil (Lol). Red symbols indicate tetraploid daughter plants (4x), while black symbols indicate diploid daughter plants (2x).

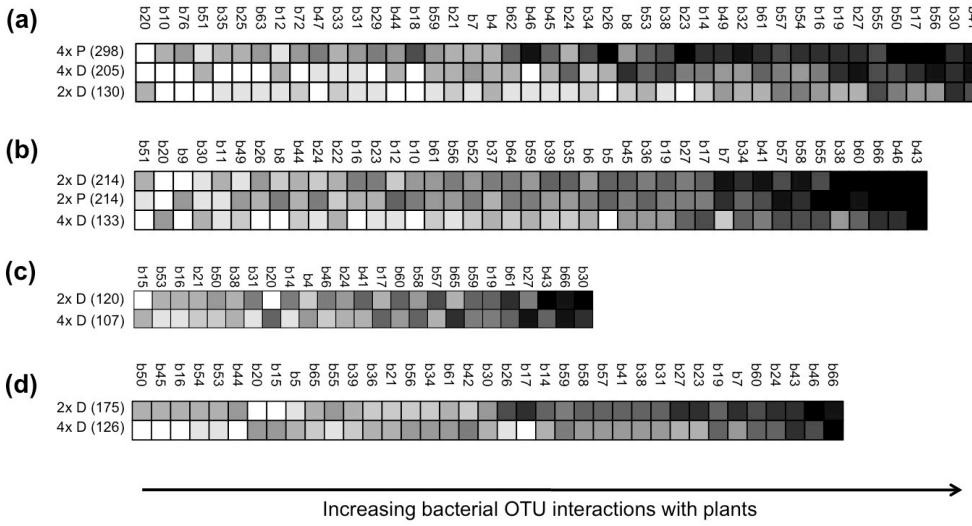


Figure 3. Interaction matrices between plant populations (rows named on left: P = parental, D = daughter) for both 4x and 2x populations and the bacterial OTUs detected (columns) in pots inoculated with soil from (a) clover-conditioned 4x site, (b) clover-conditioned 2x site, (c) lolium-conditioned 4x site, and (d) lolium-conditioned 2x site. More darkly shaded squares indicate the OTU occurred more frequently in replicates of that corresponding plant population. Numbers in parenthesis adjacent to plant population names is the sum of all the OTU occurrences within the row indicating the magnitude of the interaction with the bacterial OTU community detected. Numbers in the prefix of bacterial OTU names indicate its relative DNA fragment size in increasing order. OTUs must have occurred in at least 3 replicates of one plant population to appear in the matrix**.**

DISCUSSION

In this study we demonstrate 1) that different plant populations and generations can procure specific microbial communities and 2) that different plant populations demonstrate specific responses to the soil communities conditioned by their parental population. These results demonstrate that differences in adaptation to soil communities conditioned by their parental population can occur between populations differing in genetic characteristics such as ploidy and pedigree.
In specific, soil communities benefited native daughter plant populations in aboveground productivity more than non-native daughter plant populations, particularly in the case of the 4x population. However, after taking into account the influence of edaphic soil properties, 4x daughter plants demonstrated the most beneficial response in aboveground productivity when associating with the soil community conditioned by their parental population, while the opposite trend occurred in the 2x plants. This supports our initial hypothesis that soil feedback operates differentially depending on heritable characteristics of conspecific populations. Moreover, the 4x daughter population demonstrated greater adaptability to the soil communities of their parental population. Intriguingly this trend was not observed in the 2x population where both daughter and parental plants were similar in both associated microbial communities and performance. Two factors likely contributed to the adaptive differences between these two populations. Firstly, strong selection from interactions with soil communities on the tetraploid parental population, which did not occur in the diploid population, prior to seed collection of the daughter populations. Secondly, ploidy level of the populations, which has been observed to alter plant pathogen interactions in *T. pratense* (Metha & Swaminathan 1957; Vestad 1960; Arseniuk 1989), and adaptive characteristics and genetic variability in plants (Flagel & Wendel 2009; Parisod *et al*. 2010) could also have aided the adaptation in the 4x daughter plants.

The greater adaptability in the 4x population may support the concept that higher ploidy allows for greater adaptability to local ecosystem characteristics (Ramsey & Schemske, 2002; Flagel & Wendel, 2009). Genetic variability in the 4x population was likely higher resulting from polyploidy and may also have aided in more rapid adaption in the 4x population (Parisod *et al*. 2010). However, since we cannot separate the effect of plant pedigree from the effect of ploidy, as 4x and 2x populations did not originate from the same plant population, both ploidy and pedigree may have contributed to the observed differences between our populations.

Soil feedback on plant performance

It has been demonstrated that soils previously inhabited by conspecifics will procure a specific community of antagonistic and mutualistic organisms different from that of heterospecifics (Bever *et al.,* 1997). Such differences in soil communities subsequently can influence establishment and performance of the successive plants (Klironomos, 2002; Kardol *et al*., 2007; Petermann *et al*., 2008). In this study, we found soil feedback effects can also differentially act upon heritable differences between populations of the same species. Importantly, the influence of native soil communities on plant performance and associated microbial communities was not observed when inoculated with lolium-conditioned or sterilized soil from the same site. This demonstrates the effects are due to differences in the common history between plant populations and associating soil biota. It is possible that other soil fauna not characterized here, such as invertebrates, also contributed to feedback effects (De Deyn *et al*., 2003). However, it seems likely that in our case root endophytic symbionts were a major contributor to the observed feedback effects since *T. pratense* relies heavily upon these symbioses (Boller & Nösberger, 1987; Feng *et al*., 2003; Wagg *et al*., 2011b,c). Furthermore, both populations inoculated with their native soil associated with a more rich and unique bacterial OTU community structure, reflecting host population specific interactions that should exist if feedback is to hold clout (Freckleton & Lewis, 2006).

The positive feedback effect on the 4x plants that did not occur in the 2x plants may be due either to a build up of symbionts that function more beneficially in association with 4x relative to 2x *T. pratense* and or greater resistance in 4x than 2x plants to antagonistic interactions with soil biota. Similarly, Sudová *et al*., (2010) found no differences between ploidy levels in three herbaceous plants in the proportion of roots colonized by AMF. However, these authors report inoculation with AMF influenced growth responses differentially between cytotypes of *Aster amellus*, where the polyploid depended less on this mutualism for aboveground biomass production. The little differences in AMF colonization between 4x and 2x daughter plants could indicate 4x plants were not more efficient than 2x plants at acquiring and benefiting from AMF. However, it is difficult to speculate as to the mechanism based on root length colonization alone (see Facelli *et al*. 2009).

Hosts with higher ploidy are thought to be favoured in the co-evolution of host-parasite interactions (Nuismer & Otto, 2004) as the increase in the dosage of

resistant genes in polyploids can result in improved functional resistance to pathogens (Levin, 1983; Flagel & Wendel, 2009). In addition many previous studies report improved pathogen and pest resistance in *T. pratense* tetraploids compared to diploid progenitors (Metha & Swaminathan, 1957; Vestad, 1960; Arseniuk, 1989). Hence, the increased resistance to disease in the 4x population relative to the 2x population is perhaps the more likely explanation for the differences in soil feedback effects between the two plant populations than improved symbioses.

Adaptation in plant - soil microbial community interactions

The difference in growth response between the two generations when inoculated with the same soil community illustrates the degree of adaptive differentiation in the two cytotypes to their native soil communities. The little to no differences in growth characteristics between the 2x generations compared to the consistently large differences in characteristics between the 4x generations (nearly 1.5 fold increase in biomass) may reflect the greater ability of the 4x plants to quickly adapt to their native soil communities relative to the 2x plants. This coincides with the concept that polyploid plants have greater genetic plasticity providing them with the ability to adapt more rapidly to environmental conditions than diploid counterparts (Flagel & Wendel, 2009; Leitch & Leitch, 2008) and is perhaps a mechanism by which plants escape strong negative feedback effects. Although differences in seed quality, as indicated by seed size, can influence plant performance (eg. Halpern, 2005), this was likely not a factor in the performance between generations as seed sizes were similar (see methods). Moreover, differences observed in root associated fungal and bacterial community characteristics coincide with changes in plant performance. This parallel between specialized root associated microbial communities and plant performance provides the most parsimonious evidence for the mechanism behind the differences in responses between the two generations in the 4x population.

Similar to the differences between populations in feedback effects, such an increased adaptive response in the 4x daughter plants reflects increased efficiency in mutualistic interactions, such as AMF and N-fixing bactera, and or resistance to antagonistic interactions, such increased pathogen defense. For example, it has been previously observed that the introduced North American populations of *Hypericum perforatum* was less dependant on AMF for growth than their European congeners, which may have led to the successful establishment of this species in the

novel environment (Seifert *et al.*, 2009). However, the reduced colonization by non-AMF fungal structures as well as the reduced interactions and unique assemblages of the bacterial community with the 4x daughter plants relative to their parental plants would indicate adaptation to suppressing association with antagonistic soil microbes in this population. This further indicates the greater adaptability in the 4x population than the 2x population to their native soil communities for reasons previously mentioned and could suggest polyploids are better able to escape negative plant-soil feedback effects though increased adaptive ability.

Overall, the generational differences in performance between these two populations would suggest the common history between plants populations and their associating microbial communities has the potential to play an important role in driving adaptive diversification in plants through a plant-soil feedback loop. Our results add to the literature base that has begun to emerge demonstrating the potential for soil microbial communities to influence evolutionary processes in plant communities and the potential mechanisms that may influence ecological divergence in plants differing in heritable characteristics such as polyploidy. Such results provides the initial evidence to indicate plant-soil feedback as a mechanism involved in the ongoing evolution in plant communities in concert with other evolutionary processes such as polyploidy and breeding.

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Appendix A Substrate characteristics

Soil nutrient concentrations and pH in treatments which received unsterilized and sterilised soil inoculum. The inoculum was collected at plots with clover and Italian ryegrass each grown at two different sites (4x site and 2x site). At one site a diploid clover genotype (2x site) was grown while at the other sites a tetraploid clover genotype (4x site) was grown. It can be observed that sterilization of inocula influenced plant available N, P, K and soil pH of the overall substrate.

	Unsterilized				**Sterilized**			
	4x site		**2x site**		**4x site**		**2x site**	
Soil Characteristic	**Clover**	**Lolium**	**Clover**	**Lolium**	**Clover**	**Lolium**	**Clover**	**Lolium**
pH	7.6	7.5	7.6	7.5	7.9	8.0	7.7	8.0
Plant available nutrients ($mg \cdot kg^{-1}$)								
Water soluble NO_3- and NH_4+	72.1	71.9	72.0	68.8	53.5	55.4	58.3	56.9
P_2O_5 (CO_2-saturated water extracted)	2.65	3.60	3.40	3.35	3.00	4.10	4.05	5.30
K_2O (CO_2-saturated water extracted)	1.05	1.05	1.70	1.15	1.25	1.45	1.80	1.90
Ammonium acetate -extracted mineral nutrients ($mg \cdot kg^{-1}$)								
Ca ($\times 10^3$)	8.86	8.26	6.58	7.16	9.70	7.86	7.25	8.02
P	19.9	24.7	21.8	23.7	23.8	25.7	23.1	30.6
K	34.5	32.9	46.3	34.0	26.6	32.0	53.0	42.4
Mg	231.3	199.3	186.0	194.2	183.3	182.6	205.0	228.2

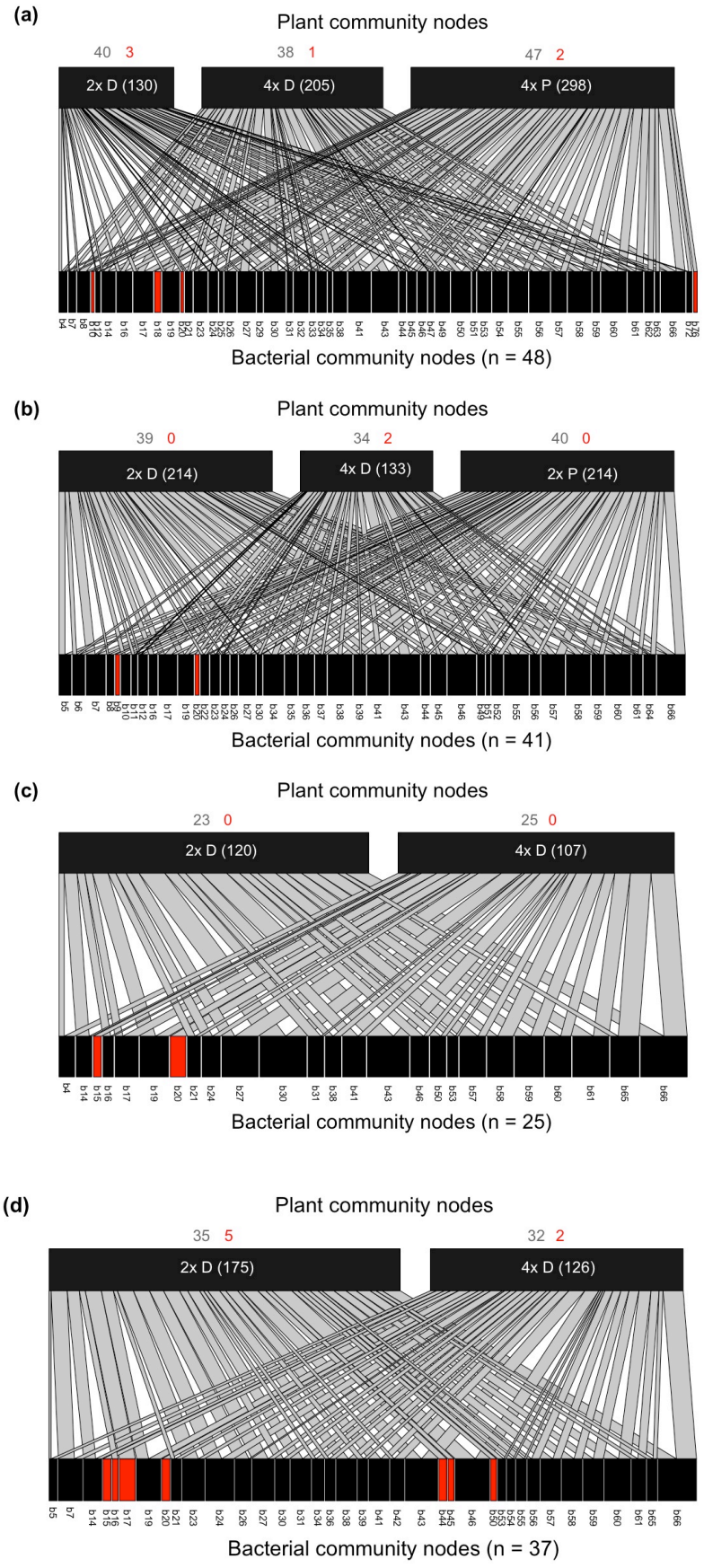
Appendix B Flowering and mortality data

Total number of flowering heads produced, their mean mass with standard errors of the mean in parentheses, and the percent mortality after 14 weeks of growth for each soil inocula origin (2x site and 4x site), history, treatment, plant ploidial level and plant generation (Gen; D = daughter, P = parental) combinations and the number of replicate pots included (N). Replicate pots excluded were due to the presence of AMF or root nodules in pots that were inoculated with sterilized soil.

Inocula factors			Plant factors					
Origin	**History**	**Treatment**	**Ploidy**	**Gen**	**N**	**Flower heads**	**Flower head mass (mg)**	**Mortality (%)**
4n	Clover	Living	4x	D	10	2	69.6 (0.7)	1.67
4n	Clover	Living	4x	P	10	0	n.a.	1.67
2n	Clover	Living	4x	D	10	3	118.6 (13.8)	0.00
4n	Lolium	Living	4x	D	10	2	47.5 (29.8)	10.0
2n	Lolium	Living	4x	D	10	3	90.8 (38.0)	5.00
4n	Clover	Living	2x	D	10	9	97.8 (18.0)	6.67
2n	Clover	Living	2x	D	10	25	129.2 (10.7)	6.67
2n	Clover	Living	2x	P	10	23	119.4 (9.1)	1.67
4n	Lolium	Living	2x	D	10	18	96.1 (12.6)	3.33
2n	Lolium	Living	2x	D	10	14	111.4 (15.5)	8.33
4n	Clover	Sterile	4x	D	10	0	n.a.	5.00
4n	Clover	Sterile	4x	P	10	0	n.a.	1.67
2n	Clover	Sterile	4x	D	9	0	n.a.	8.33
4n	Lolium	Sterile	4x	D	10	0	n.a.	8.33
2n	Lolium	Sterile	4x	D	8	0	n.a.	0.00
4n	Clover	Sterile	2x	D	10	0	n.a.	35.0
2n	Clover	Sterile	2x	D	8	0	n.a.	33.3
2n	Clover	Sterile	2x	P	8	1	52.0	3.33
4n	Lolium	Sterile	2x	D	10	0	n.a.	25.0
2n	Lolium	Sterile	2x	D	8	0	n.a.	33.3

Note: n.a. = not available

Appendix C Bipartite networks of bacterial OTU interactions with plant populations



Appendix C. Bipartite network illustrating the bacterial OTU associations to plant populations when inoculated with clover-conditioned soil originating from (a) the 4x site or (b) the 2x site and lolium-conditioned soil originating from either (c) the 4x site or (d) the 2x site. Numbers in parentheses are the sum of OTU occurrences and corresponds to the width of the plant population nodes. Bacterial OTU nodes highlighted in red indicate association with a single plant population. Numbers above of each plant population is the number of links following the same colour coding. Width of the connection is the relative frequency in which the OTU was present in replicates of that population. OTUs must have occurred in at least 3 replicates of one plant population to simplify the web. Numbers in the prefix of bacterial node names indicate its relative DNA fragment size (b1 = OTU with smallest DNA fragment). Bacterial OTU nodes are ordered in increasing fragment size from left to right. Note: P = parental plants, D= daughter plants.

General Discussion

Biodiversity provides a number of important ecosystem services to human society (Hooper *et al*. 2005). This includes the provision of food, promotion of primary productivity, a more efficient cycling of nutrients, resistance to drought, and climate change. This realization has created concern for the future functioning of ecosystems as global biodiversity loss exceeds limits thought to have detrimental consequences for ecosystems processes (Rockström *et al*. 2009). Despite the vast and relatively unexplored diversity in belowground terrestrial ecosystems compared to their aboveground counterparts (Balvanera *et al*. 2006), soil communities are becoming recognized as an influential element in maintaining the functioning of terrestrial ecosystems (Bardgett & Wardle 2010). Yet, the effects of soil biodiversity loss due to anthropogenic activities and the consequences it holds for maintaining ecosystem functioning have only begun to be elucidated.

This dissertation provides empirical evidence to demonstrate the multiple roles of soil of biodiversity in shaping ecosystem characteristics and maintaining its functioning. Both species richness of mycorrhizal fungi and the abiotic characteristics of soils were demonstrated to be important for improving plant species coexistence and productivity of plant communities (Chapters 1 & 2). However, the mechanism, such as niche differentiation, by which mycorrhizal species complementarily function to improve the productivity of plant communities remains elusive (Chapters 3). Although mycorrhizal fungi are key organisms within terrestrial ecosystems, they are yet only a fraction of the biodiversity belowground. The data presented within this thesis also demonstrates the destructive depletion of organisms comprising soil biodiversity can consequently suppress the functioning of the ecosystem as a whole (Chapters 4). The results show the necessity for maintaining soil biodiversity to achieving optimal functioning of multiple ecosystem process, such as nutrient retention and plant diversity. Although soil biodiversity is a critical driver of ecosystem processes, plant communities and their functional, physiological, and genetic characterises shape soil communities resulting in ongoing co-evolution between above-belowground communities through feedback mechanisms (Chapter 5). This relatively unexplored nature of above-belowground communities surely holds exciting future discoveries for both basic and applied research.

Plant communities span in both above- and belowground ecosystems acting as a bridge by which belowground ecosystems shape aboveground ecosystems and vice versa (van der Putten *et al*. 2004; Bardgett & Wardle 2010). For instance, the diversity and composition of plant communities can influence the diversity and composition of soil communities (Johnson *et al*. 2003; Bezemer *et al*. 2010) and soil community composition and diversity can influence the aboveground plant community characteristics (van der Heijden *et al*. 1998; Scherber *et al*. 2010; Maron *et al*. 2011). However, mechanisms behind the community effects in one trophic group that influence the functioning of the other trophic group is yet to be fully unravelled.

Species richness and compositional influences on the functioning of communities are a cumulative result of the mutalistic and antagonistic interactions among the species comprising the community (Loreau & Hector 2001). These interactions result from the realized niches species occupy within a community context; such as spatial and temporal resource use. In particular, resource availability and heterogeneity can influence the functioning of more diverse communities by promoting mutualistic or antagonistic interactions depending on the quality of the environment (Thrall *et al*. 2007). For example, within plant communities increased resource availability may result in increased antagonistic / competitive interactions among species (Hautier *et al*. 2009), while heterogeneity may promote complementarity by increasing available niche space and reducing competition (Hutchings *et al*. 2003; Ashton *et al*. 2010; Jousett *et al*. 2011). These abiotic characteristics that influence biotic interactions may ultimately determine the degree of functional redundancy and complementarity observed within more species rich communities (Joussett *et al*. 2011; Wagg *et al*. 2011b). Therefore, abiotic characteristics of an environment can be a strong determinant of the presence and functioning of organisms within communities that drive above-belowground relationships. This has been a major theme throughout this dissertation and illustrated using grass (*Lolium multiflorum*) – legume (*Trifolium pratense*) - AMF model communities under differing soil regimes (Fig. 1; see Chapters 1-3 for AMF species used).

Plant-AMF communities are tightly interlinked and their relationships can be influenced by abiotic factors. For instance, plant communities can be strongly influenced by arbuscular mycorrhizal fungi (AMF) through their provision of plant inaccessible soil resources (Fig. 1a; Reynolds *et al*. 2003; Smith *et al*. 2009).

Conversely, AMF depend solely on their plant hosts for sources of C attained from photosynthetically derived carbohydrates (Fig. 1b; Pfeffer *et al.* 1999). This bidirectional relationship can result in a feedback loop between plants that shape their belowground communities that ultimately feedback to influence the performance of succeeding and neighbouring plants (Fig. 1a-b-a; Janzen 1970; Connel 1971; Bever *et al*. 1997).

This intimate above-belowground relationship can function dynamically with changes in environmental and abiotic conditions such as through nutrient inputs (Johnson 2010). For example, plant community characteristics can be shaped by soil abiotic factors (Fig. 1c), and thus the relationship with associated AMF (Hoeksema *et al*. 2010). Additionally, as in plant communities, AMF communities are likewise influenced by soil abiotic factors (Fig. 1d) that shape the assemblage of AMF species (Lekberg *et al*. 2007; Oehl *et al*. 2010). These abiotic controls on plant and AMF communities ultimately can determine the functioning of AMF – plant community relationships. For instance, soils with high levels of plant available nutrients often result in neutral to negative effects of AMF on plant productivity (Hoeksema *et al*. 2010; Johnson 2010). This effect may occur if plants do not require AMF partners for acquiring resources to achieve optimal growth and thus AMF associations may provide no benefit or incur a cost to the plant (Johnson 2010). Thus, the alteration of soil abiotic characteristics indirectly influences plant community characteristics via altered trophic interactions (eg. Fig. 1d,a). These are some of the major mechanisms that drive the above-belowground interactions discussed that shape terrestrial ecosystems.

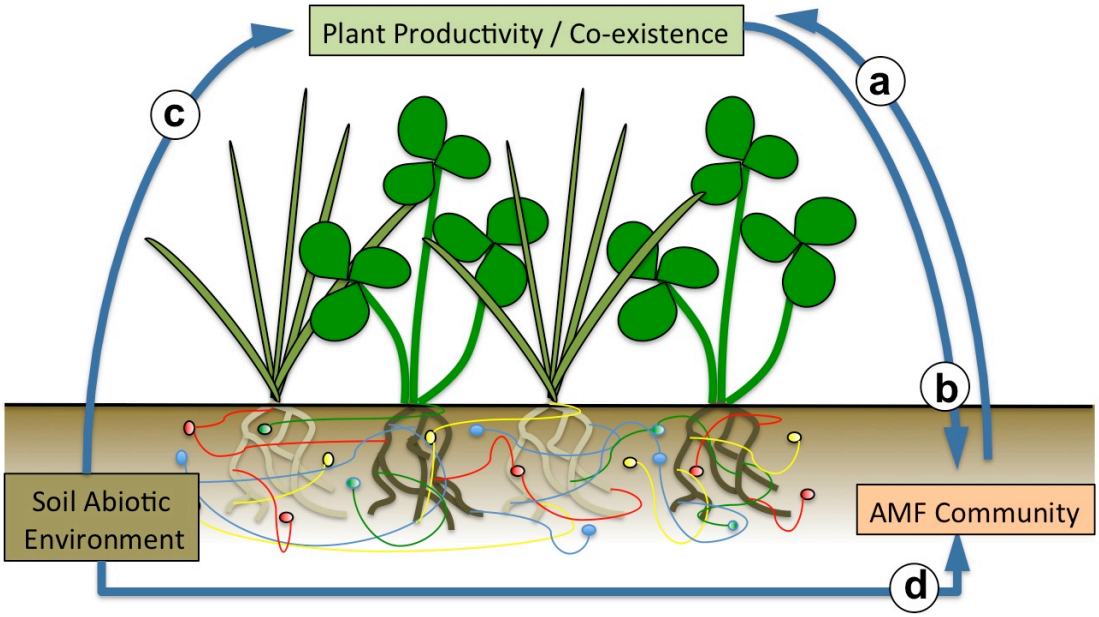


Figure 1. Schematic of interactions between plant and mycorrhizal fungal communities driven by (a) The ability for AMF dependant plants to utilize soil resources is driven by the AMF community characteristics (van der Heijden *et al*. 1998). (b) Plant community characteristics control AMF community characteristics (Johnson *et al*. 2003). (c) Soil abiotic characteristics control the productivity and diversity within a plant community (Hautier *et al*. 2009). (d) Abiotic soil characteristics that control the AMF community assembly and functioning (Lekberg *et al*. 2007; Johnson 2010).

Mycorrhizal communities drive the functioning of plant communities

The addition of plant species in grasslands has been shown to coincide with increases in net plant productivity (eg. Tilman *et al*. 1996; Hector *et al*. 1999; Tilman *et al*. 2001). If this effect is driven by the contribution of each additional plant species present, it requires that the additional plant species is able to capture the resource margin so that it is able to contribute to the productivity of the community (Loreau & Hector 2001). Differences among plant species in their ability to utilize different resource pools has previously been observed to be one mechanism by which sympatric species coexist via disparate niches (McKaine *et al*. 2002; Dimitrakopoulos & Schmid 2004; Harrison *et al*. 2007) and contribute to the complementarity and overyielding in plant species mixtures (Ashton *et al*. 2010).

The fact that the presence of AMF and the richness of AMF species can improve plant diversity and productivity (eg. van der Heijden *et al*. 1998) indicates they are key in shaping relationships between plant species richness and plant productivity in grasslands. This is because AMF provide soil resources otherwise inaccessible to some plants (Smith & Read 2008), thereby altering the available resource niche space (Klironomos *et al*. 2000). Moreover, this mode by which plants

acquire soil resources can be imbalanced among plant species resulting in a shift in the competitive balance between species in the presence of AMF (Fitter 1977, Hartnett *et al.* 1993, Scheublin *et al*. 2007; Collins & Foster 2009), therefore potentially altering the biodiversity effects in plant communities. Klironomos *et al*. (2000) nicely illustrate the addition of an AMF species alters the resource-niche space utilized by plant communities. They found the presence of an AMF resulted in a positive asymptotic relationship between plant richness and productivity where plant productivity became saturated at low levels of plant species richness in comparison to the absence of AMF, which resulted in a linear relationship; indicative of a change from a complementarity to a selection driven biodiversity effect in the presence of AMF (see Fig. 1c in General Introduction and Bell *et al*. 2005).

What is missing from this above-belowground relationship, however, is whether or not species richness in the AMF community alters plant coexistence and resulting productivity in plant mixtures. This was the main aim of Chapter 1 of this dissertation as natural plant-AMF communities rarely consist of single species. Furthermore, if AMF taxa differ in the realized niche, such that they provide different benefits to each of the plant species in the mixture, a greater AMF species rich community could result in greater coexistence and reduced niche overlap between the plants and thus greater complementarity in the plant community. Hence, the communal functioning of an AMF community can shift the functioning of plant community between scenarios depicted in Fig. 1b and c presented in the General Introduction.

Using data from Chapter 1, it was possible to illustrate the changes in the functioning of plant communities under differing soil fertility and AMF community regimes by partitioning the net biodiversity effect into the complementarity and selection effects (*sensu* Loreau & Hector 2001). As reported in Chapter 1, a more rich AMF community altered the competitive interaction in the grass-legume plant communities in favour of the legume. However, as reflected in the relative yield total (RYT), AMF did not necessarily increase plant productivity and plant complementarity. (Fig. 2a,b; also see Fig. 2e,f in Chapter 1). Only 1 of the 6 plant combinations grown in both soils demonstrated the greatest plant complementarity effect when inoculated with the mixture of all four AMF (Fig. 2a, 25% *T. pratense* in mixture in sandy soil). Similarly, the selection effect in the plant community was also dependant on the combinations of soil type and presence of AMF in the various plant community mixtures (Fig. 2c,d; not determined in Chapter 1). Generally, the selection

effect was neutral to negative, demonstrating the depressions in *T. pratense* growth that were not always countered by improved *L. multiflorum* growth in plant mixtures. The selection effect was only positive in the absence of AMF in the fertile soil and increasingly so with *T. pratense* being more common within the mixtures, reflecting the large benefits to *L. multiflorum* growth, while changes in *T. pratense* biomass accounted for little of the productivity in the plant mixtures (Fig. 2d).

Overall, whether or not plant communities showed a positive net biodiversity effect as a result of a stronger complementarity or selection effect is dependant on both the abiotic and biotic soil characteristics (Fig. 2e,f). Specificly, the presence of AMF altered the competitive interaction between these two plants species and generally improved the net biodiversity effects within the sandy soil (Fig. 2e). This was largely due to the influence of AMF on the complementarity effect in the majority of these plant mixtures (Fig. 2a; Fig. 3). Conversely, in the fertile soil the net biodiversity effect was suppressed in the presence of AMF (Fig. 1f) that largely reflected the selection effect (Fig. 1d; Fig. 3). However, in one case in the fertile soil (*G. intraradices*), a positive net effect resulted from this fungus improving the complementarity in the plant communities. These scenarios nicely demonstrate the biotic and abiotic components of soils that control the functioning of plant communities illustrated in Fig 1. Furthermore, the data show a greater AMF richness may not always improve the complementarity within the associated plant community, highlighting the context dependency of the functioning of these above-belowground communities (Hoeksema *et al*. 2010).

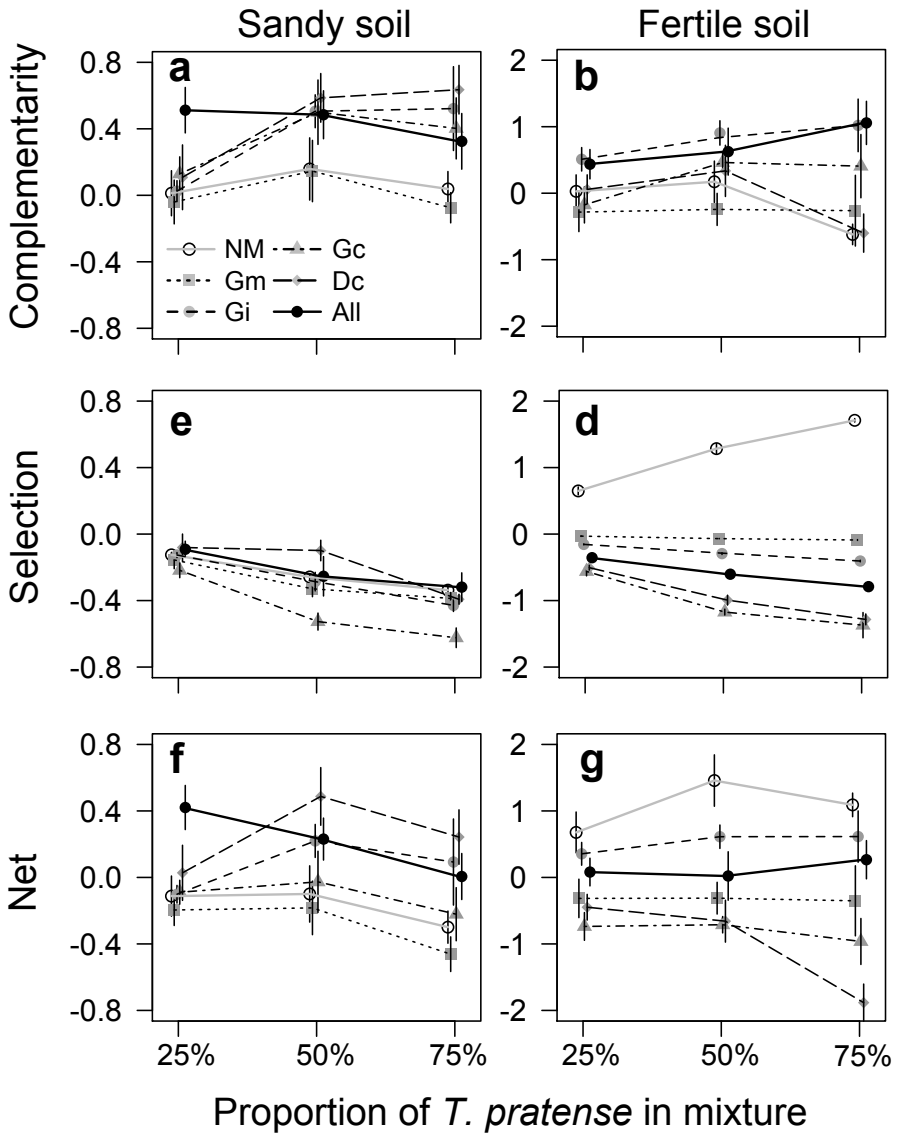


Figure 2. Plant biodiversity effect means (in g) and standard errors are shown for each planting mixture for both 'sandy' (left panels) and 'fertile (right panels) substrates and for each AMF inocula treatment: NM= non-mycorrhizal, Gm = *G. mosseae*, Gi = *G. intraradices*, Gc = *G. claroideum*, Dc = *D. celata*, and All = mixture of all four AMF fungi. Lines connecting means are for clarity and illustrate trends. Note the complementarity effect (CE) reflects the same trend as the relative yield total (RYT) in Chapter 2 only on a different scale, such that CE = (RYT-1) x (mean of plant monocultures); see Loreau & Hector (2001) for details.

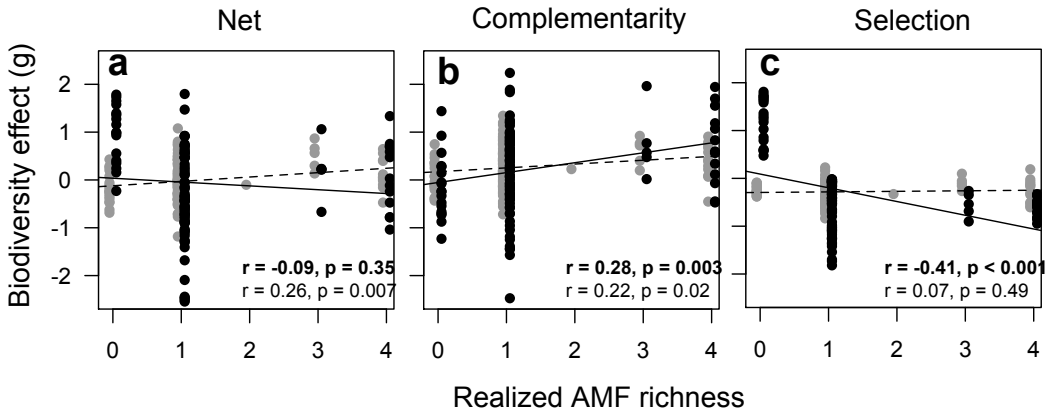


Figure 3. Correlations (Pearson's) between AMF realized richness and biodiversity effects in the plant communities (pooled for all three plant mixtures) in both the fertile soil (black dots, solid line, values in above in bold) and sandy soil (grey dots, dashed line, values below).

The functioning of species rich mycorrhizal communities

In previous studies, increasing species richness of AMF has been shown to improve the productivity of the plant community (van der Heijden *et al*. 1998; Vogelsang *et al*. 2006; Maherali & Klironomos 2007). This is often interpreted as complementary in functioning of the of the AMF species comprising the community, such that each additional AMF species improves the ability of the AMF community to support the aboveground plant community. However, other studies illustrate a more rich community of AMF provides similar benefits as the single most beneficial AMF species comprising the community (eg. Jansa *et al*. 2008; Chapter 2). Subsequently, this has been associated with the occurrence of a selection effect (eg. Vogelsang *et al*. 2006). The difference in interpretations often depend on whether it is assessed if a more rich AMF communities have higher productivity than the single AMF species treatments that comprise the AMF rich community; essentially testing for "transgressive overyielding" effects, versus whether more rich AMF communities have a higher productivity than the average of all single AMF species treatments; the net biodiversity effect *sensu* Loreau & Hector (2001). Although transgressive overyielding would likely indicate the presence of a stronger complementarity effect than a selection effect, the lack of transgresive overyielding does not imply complementarity did not occur (see Hector *et al*. 2002). An additional complication in determining the mechanisms behind richness-ecosystem functioning relationships in some of these studies results because not all AMF species present in mixtures are assessed as monocultures (eg. van der Heijden *et al*. 1998; Maherali & Klironomos 2007). Thus, it is impossible to infer whether the functioning of a more rich AMF

community occurred as a result a net biodiversity effect or a sampling–probability effect.

Similar to past studies, Chapters 2 demonstrates that increasing AMF species richness improved biomass production in plant communities. This was due to the large beneficial effects on *T. pratense*, the more AMF dependant plant. However, when more rich AMF communities were compared to the effects of each of the species in monoculture that comprised the community often effects were similar, indicating no transgressive overyielding. By using novel molecular tools (Thonar *et al*. 2012) to estimate the contribution of each AMF in the mixture to the response in aboveground biomass we demonstrated similar effects between AMF mixtures and monocultures are not always due to the presence of a particular AMF species. Thus illustrating that non-transgressive overyielding effects may arise from either strong selection or complementarity effects in the associated AMF community. Furthermore, the data revealed positive relationships between AMF species richness and plant productivity can be driven by either a complementarity or selection effect (Chapter 2; Wagg et al. 2011b). The combination of the AMF species comprising the community was an important factor behind the observed biodiversity effects, indicating competitive interactions among AMF influences the overall functioning the AMF community in parallel with previous studies (Bever *et al*. 2009; Bennett & Bever 2009). The effects of soil type on the functioning of more species rich AMF communities, however, was most striking. The complementarity effect drove the functioning of the AMF community in the fertile soil, while the selection effect drove the functioning of more AMF rich communities in the sandy soil and resulted in richness–productivity relationships reminiscent of those illustrated in Fig. 1 of the General Introduction and Bell *et al*. (2005).

These results highlight the necessity to assess the contribution of AMF species that comprise a community when assessing the mechanisms behind the functioning of species rich communities. However, unlike in plant communities, the direct contribution of each species within the belowground community to the overall observed effect aboveground is difficult to discern since contributions of each AMF species to plant productivity cannot be directly measured. To overcome this, novel molecular tools are needed to decipher the functioning of soil organisms within a community context. The use of DNA abundance to infer functionality of a species within a community is one feasible possibility as demonstrated in Chapter 2. However, such methods are not without caveats and assumptions that require further

testing. In particular, whether DNA abundance within roots reflects the functioning of the fungus within the soil and whether relationships between DNA abundance and function change through time.

Controls on the functioning of mycorrhizal communities

The difference in the effects of increasing species richness of AMF on plant productivity between the two soil types in Chapter 2 illustrates context dependent controls on the functioning of the AMF community. The more fertile soil may have provided more abundant and heterogeneous resources (see Appendix A in Chapter 2) for AMF species to differentially utilize, thereby reducing niche overlap and allowing for greater coexistence (Fig. 1d). Additionally, the more fertile soil improved the overall productivity of the plant community, particularly in *L. multiflorum,* thus perhaps resulting in a greater allocation of photosynthetically derived C belowground to support a more diverse AMF community (Fig. 1b). However, it should be noted that although DNA abundance of AMF and the diversity (Shannon index) of AMF detected in roots were greater in the fertile soil neither measure differed significantly between soil types (DNA abundance $F_{1,9} = 2.27$, $P = 0.17$; Shannon diversity $F_{1,9} = 4.59$, $P = 0.06$). Similar to the AMF and soil type controls on the functioning of the plant community (Fig. 1a,c), these results indicate trophic interactions and abiotic factors likewise control the functioning of the AMF community (Fig. 1b,d). These abiotic and biotic characteristics must therefore shape the realized niches that each AMF species is able to occupy within a community of AMF (eg. Fig. 4).

The reduction of competition among AMF may improve their ability to promote plant productivity (Bennett & Bever 2009) and may occur via spatial niche segregation (Bever *et al*. 2009). Several studies report the occurrence and abundance of various AMF species within natural systems are regulated by combinations of temporal and abiotic factors, such as seasonality and abiotic soil characteristics (Lekberg *et al*. 2007; Dumbrell *et al*. 2009; Oehl *et al*. 2010). Additionally, a number of studies have illustrated the occurrence of AMF host preference in natural systems (Croll *et al*. 2008; Sýkorová *et al*. 2007). This could suggest complementarity in AMF functioning could result from spatial niche segregation via host and abiotic preferences (eg. Fig. 4E; Chapter 3).

Although, niche segregation via host and soil patch preferences occurred in the experimental pots of Chapter 3, it did not seem to be a mechanism by which AMF are able to provide greater benefits to plant productivity (Chapter 3). Although, the

data set was not extensive, and thus limiting any concluding biological inferences between niche differentiation and functioning of AMF communities, there are a number of possible scenarios that could shape the spatial niche differentiation among AMF in plant hosts and soil patches. For example, host preference may only occur as a result of coevolved traits between AMF and plant host species combinations that are not necessarily associated with the productivity of the plant or the soil (Fig. 4B). Alternatively, host preference may occur as a result of associating with the plant that is the most productive within given conditions such that the host readily provides a source of carbon (Fig. 4C; Kiers *et al*. 2011). In this scenario, host preference is beneficial for the fungus and less for the host. The results of Chapter 4, however, suggest soil abiotic factors are a strong determinant of whether host preference occurs (Fig. 4D). This parallels similar findings that AMF community structure, although somewhat stochastic, is driven by abiotic soil characteristics (Dumbrell *et al*. 2009; Oehl *et al.* 2010) and perhaps overrides any host preference effect (Schnoor *et al*. 2011). All of these scenarios, unless governed solely by AMF-plant coevolved relationships or strict abiotic constraints, are perhaps more likely a result of optimal foraging strategies by which the fungus and plant optimize the efficiency of their interaction to reduce input of resource in limited supply while optimizing required resources gained (Kiers *et al*. 2011).

The inner workings of these 'bottom-up' (abiotic resource abundance and heterogeneity) and 'top-down' (trophic interaction) controls on the plant-AMF community are slowly being unravelled; yet the realized niches various AMF occupy to function complementarily are quantifiably elusive. Although the site of interaction between these below-aboveground communities occurs within roots, the functional niches AMF occupy may occur within the soil matrix and thus are not elucidated by assessing community structure in roots alone. Additionally, past studies describe the differences in functional traits among AMF fungi such as the promotion of growth and nutrient uptake in hosts (Klironomos 2003; Sikes *et al*. 2009), resource acquisition strategies (Jansa *et al*. 2005; Thonar *et al*. 2010), and pathogen protection (Maherali & Klironomos 2007; Sikes *et al*. 2010). These differences in functional characteristics are perhaps more important than spatial niche differentiation via host and abiotic preferences as the mechanisms by which AMF communities function complementarily to provide greater services to their plant hosts (Koide 2000).

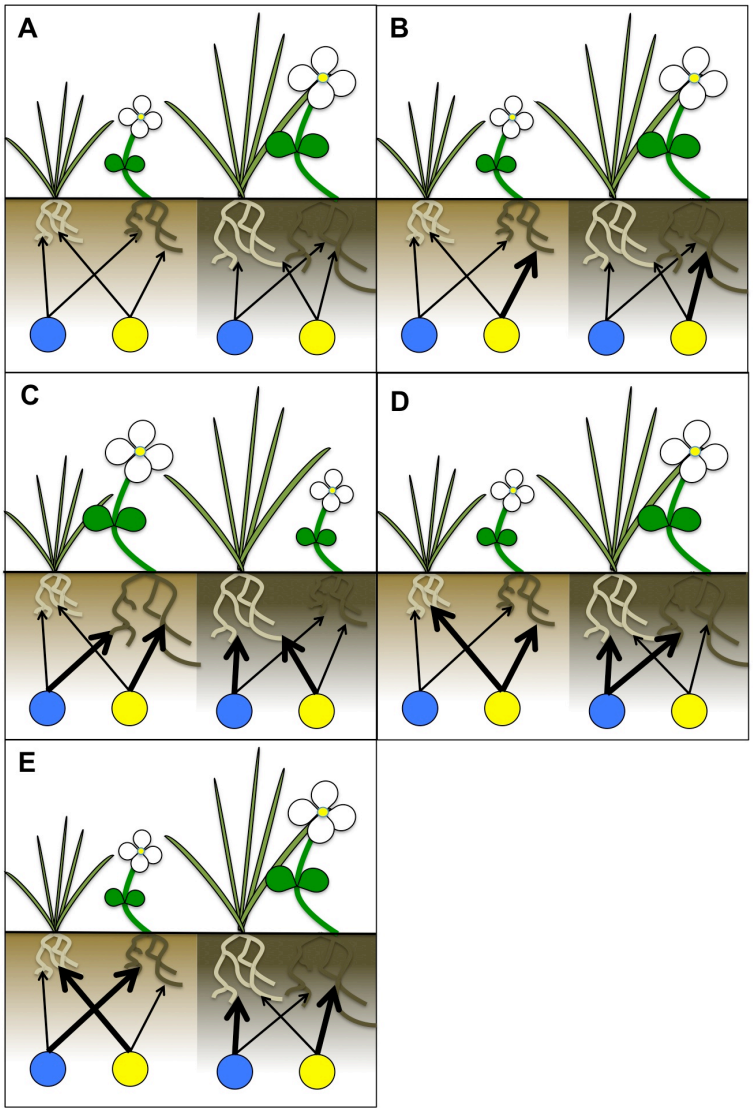


Figure 4. Illustrations of the various scenarios by which plant and soil controls can determine the realized niche of AMF. Coloured circles represent different AMF species, the thickness of arrows indicates the strength of the association (a combination of AMF resources invested in the plant and abundance within the plant roots). The background colour indicates soil type and the size of the plants indicates their productivity. The scenarios are as follows: (A) the null hypothesis that interactions are stochastic despite soil and plant characteristics, (B) host specialists (yellow) and generalists (blue) occur despite differences in soil characteristics (indicative of highly conserved evolution for or against co-interdependence, respectively), (C) AMF associations depend on soil characteristics, and thus host performance, alone (D) the functioning of AMF is driven by the soil characteristics, despite host performance. (E) An example of how soil and host preference could be facilitated by optimal niche segregation.

Depletion of soil biodiversity and ecosystem sustainability

Soils harbour a broad range of organisms and functional groups (symbionts, mutualists, predators, pathogens, saprophytes, necrotrophs, nitrifiers, denitrifiers etc.) that interact to produce a functionally complex ecosystem that influence numerous soil processes to shape terrestrial ecosystems (Bardgett & Wardel 2010). This highly diverse and complex soil ecosystem can affect plant productivity and diversity as well as numerous additional ecosystem processes that agriculture depends upon, such as nutrient cycling, soil structure and biological control (Matson *et al*. 1997; Altieri 1999; Kibblewhite *et al*. 2008; van Elsas *et al*. 2012). Typically, functional diversity in meso and micro soil communities is more commonly seen as the important driver than richness of species *per se* (Heemsbergen *et al*. 2004; Gravel *et al*. 2010; Jousset *et al*. 2011) and is the focus of discussions on functional redundancy in soil ecosystems (Allison & Martiny 2008). However, many studies addressing the functioning of soil biodiversity generally assess the functioning of species richness within a single trophic or guild of organisms (such as in Chapter 3), while the greatest functional diversity in soils occurs in vertical diversity (across trophic groups and functional guilds) as opposed to horizontal diversity (with a functional guild such as mycorrhizal fungi). Furthermore, considering the diversity of ecosystem processes soil organisms are involved in, it is only logical to consider the importance of their biodiversity in multifunctional perspective.

Chapter 4 provides the first comprehensive look at the multifunctionality of soil biodiversity in a holistic ecosystem approach. The results illustrate soil biodiversity as a key driver behind multiple below- and aboveground processes such as N turnover, nutrient retention, and aboveground productivity and diversity. The filtration of soil biota based on size is a wholesale removal and suppression of trophic groups and functional guilds of soil organisms and represents the reduction the vertical biodiversity. Most ecosystem functions declined rapidly with the destruction of soil communities using fine scale filtration (50-25 μm and smaller), corresponding to the filter sizes where fungal and bacterial communities were strongly suppressed. However, reductions at larger filter sizes (250 μm) most ecosystems functions were similar to the most complete soil community inoculated (5000 μm). Although this could indicate soil microbial biodiversity as the primary support system for the ecosystem processes assessed, it should not be considered evidence for the redundancy in function of higher organisms. For example, the greatest proportion of variation in ecosystem multifunctionality was explained by the filter size of the soil

organisms inoculated (Chapter 4). This demonstrates that the suppression of fungal and bacterial communities alone were not the only drivers behind ecosystem functions. In particular, the reduction in size of the organisms inoculated contributed to the decline in organic matter decomposition and nitrogen turnover. This parallels previous findings pointing to the importance of the functioning of detritivore soil invertebrate faunal communities (Laakso & Setälä 1999; Laakso *et al*. 2000). Moreover, it is likely that the combined functioning of microbes and soil invertebrates drive decomposition and nitrogen turnover (Eisenhauer *et al*. 2010; Eisenhauer 2012).

Overall, the depletion of soil biodiversity through chronic perturbations from changing climate combined with anthropogenic mechanical and chemical alterations of the soil may lead to the failure of the ecological process soil organism support. The implications of such prolonged suppression of soil biodiversity through land mismanagement combined with climatic changes, such as prolonged periods of drought, are already thought to be the reagents behind the desertification process that leads to major agricultural and socioeconomic problems (McClure 1998; Doran 2002; Schubert *et al*. 2004; Reynolds *et al*. 2007). Furthermore, soil microbial biomass and plant productivity are positively correlated on a global scale across biomes, with desert ecosystems occurring at the lower end of the spectrum and tropical forests at the opposite end (Fierer *et al*. 2009). Although this illustrates the interconnectedness among belowground biota, aboveground biota, and abiotic environment, it is difficult, if not impossible, to tease apart causal inference among these factors that drive above belowground ecosystem processes in natural ecosystems. As illustrated in Fig. 1 and throughout this dissertation these factors are tightly interlinked and their relationships are likely sensitive to changes in one of these vertexes (Hillebrand & Matthiessen 2009).

Several reports have shown that the available nice space for soil organisms can be improved by increasing organic carbon content of soils, increasing plant diversity, crop rotations and fallow seasons (Waldrop *et al*. 2006; Mäder *et al*. 2002). Thus, such measures are therefore potentially key aspects in restoration efforts in view of the strong link between soil biodiversity and ecosystem multifunctionality shown in this dissertation. Furthermore, the reintroduction of soil biota, such as mycorrhizal fungi, into previously degraded soils has promising potential for reviving soil fertility (Requena *et al*. 2001).

The ongoing co-evolution between plant and soil communities

Throughout this dissertation I have illustrated the tightly interconnected relationship between plant communities and their associated belowground communities in which one community can shape characteristics in the other (Fig. 1). This bidirectional relationship may not only shape the composition and functioning of the above and belowground communities, but may also incur evolutionary processes through a plant-soil feedback loop (Chapter 5). The results of the work presented in Chapter 5 demonstrate the strength and direction of plant-soil feedback effects can be driven by heritable plant traits and may have consequences for the performance of the progeny through adaptive interactions with soil communities conditioned by their parental population. The stark differences between the two populations of *T. pratense* when comparing parental and progeny performance illustrate differences between these two populations in the co-evolution between plants and associated soil communities. It is possible that constrained biotope space belowground in the small pots in this experiment may have amplified negative effects of soil communities by forcing roots to occur in greater density than would naturally occur in the field, thereby favouring pathogenic organisms by increased host availability and transmission (Freckleton & Lewis 2006; Thrall *et al*. 2007) or increased antagonistic interactions between soil microbes and plants for soil resources (Kaye & Hart 1997; Dunn *et al*. 2006). Nonetheless, these effects should have been relatively ubiquitous across treatments and between generations. Two factors likely contributed to the adaptive responses in these two populations. Firstly, strong selection from interactions with soil communities on the tetraploid parental population that did not occur in the diploid population over the three years the parental plants established in soils before seed was collected. Secondly, the ploidy level of the populations, which is well known to alter plant pathogen interactions in *T. pratense* (Metha & Swaminathan 1957; Vestad 1960; Arseniuk 1989) and adaptive characteristics in plants (Flagel & Wendel 2009; Parisod *et al*. 2010).

The first instance is indicative of soil community specialization on the tetraploid parental plants through either the composition of the community of soil organisms or the adaptation of the soil microbes to function optimally with this host monoculture, synonymous with host density-dependant pathogen relationships (Arneberg *et al*. 1998; Freckleton & Lewis 2006). This is perhaps also the mechanism behind negative feedback effects of the diploid population grown in association with its parental soil community. Local adaptation of plants to native soil

communities can arise from a build up of soil biota that is more specialized to function in association with a specific plant host species. This build-up of specialized associations between soil organisms and plant hosts can vary from antagonistic to mutualistic, thereby altering the fitness of the associated plant. Both positive and negative soil feedbacks likely occur in natural ecosystems, however, their net effect on the overall performance of individual plant species is typically negative (eg. van der Putten *et al*. 1993; Mills & Bever 1998; Bever *et al*. 2002; Peterman *et al*. 2008; Harrison & Bardgett 2010). This would incur that plant species are in essence loosing the ability to dominate within an ecosystem as a result of negative feedback and can be driven to rarity if unable to escape strong negative feedback effects (Klironomos 2002). This should favour the evolution of plant characteristics that allow for plants to escape negative feedback effects, such as increased dispersal of progeny or selection on traits involved in defence and symbiosis. However, the ability for selection to improve characteristics by which plants are able to escape strong negative feedback is dependent upon the genetic variation within a specific population, which may be low in some populations. This could be the case in Chapter 5 as both plant populations were generated initially from a small number of individuals. In addition, the evolution in plant traits through selective pressure alone may be too slow to maintain an advantage in the arms race between plant host and soil microbial pathogens, as generational turnover is likely much more rapid in soil microbes than plants.

The role of plant polyploidy and heritable traits that drive adaptability between generations is perhaps less understood and particularly so in understanding plant-soil community interactions (te Beest *et al*. 2012). Genome duplication events, or polyploidy, may be one mechanism by which genetic variation is increased and aid in a rapid adaptation to reduce negative plant-soil feedback. Polyploidy, however, is one aspect that is not fully understood and has mystified plant evolutionary ecologists due to the high frequency in which polyploidy occurs and the frequent ecological differentiation from progenitor populations in natural environments (Husband & Schemske 1998; Baack 2004; Baack & Stanton 2005; Mandakova & Munzbergova 2006; Buggs & Pannell 2007; Schönswetter *et al*. 2007; Ramsey 2011). Yet, little is known as to exactly how genome duplication in autopolyploids improves the environmental adaptability in plants as little differences in the functional genes between the autopolyploid and their progenitor occur (Parisod *et al*. 2010). Although, some morphological characteristics of polyploids resulting directly from genome

duplication may correspond to traits that are beneficial under certain environmental characteristics, recent studies have shown such characteristics are likely to have been amplified by post polypoloidization adaptation rather than simply gene duplication alone. This seems to indicate that altered selective pressure post genome duplication to be the key (Maherali *et al*. 2009; Ramsey 2011). Overall, the lack of current understanding as to the mechanisms as to why such ecological differentiation phenomena occur between polyploids and their progenitors is surprising since polyploidy has been of utmost importance and exploited in crop production to improve desired characteristics (Ramsey & Schemske 2002; Udall & Wendel 2006), albeit with unknown ecological consequences.

Conclusions

In this dissertation I have disclosed empirical evidence illustrating that the diversity of soil organisms and their functioning, both at the species level within a guild (horizontal diversity) or across trophic and functional guilds (vertical diversity), hold important roles for maintaining ecosystem properties, particularly for Swiss agricultural grasslands. This supports concepts that sustaining levels of soil biodiversity is desirable for achieving optimal functioning of multiple ecosystem processes (Chapter 4). Throughout the first sections of the dissertation the functioning of soil biodiversity and the interactions among species in both above (plant) and belowground (AMF) communities are interlinked through their trophic interactions and shift dynamically with changes in abiotic conditions illustrated in Fig. 1 (Chapters 1-3). Abiotic factors were some of the strongest determinants of the functioning of both plant and AMF communities (Chapter 1), and more specifically the mechanisms behind how a more AMF rich community can improve primary productivity (Chapter 2 & 3).

What can be concluded from the studies comprising this dissertation is that environmental factors (here abiotic), the functioning of species within a community, and the functioning of communities between trophic groups and functional guilds are all interconnected (Hillebrand & Matthiessen 2009). Resultantly, the loss of biodiversity, either above or belowground, or alteration of environmental factors (induced either by natural processes or anthropogenic activities) will influence the biodiversity of soils, their functioning, and the maintenance of ecological processes that support modern society. Nonetheless, the roles of soil biodiversity and the intricate workings within this complex ecosystem are by far fully understood in how

they have shaped and maintain terrestrial ecosystems. Yet, with the onset and increasingly rapid development of technology, unravelling the intricacies of the functioning of highly diverse and complex soil ecosystems within natural ecosystems will be an ever-expanding field of ecological research with implications for land management practices, improving food security, and ecological restoration.

"*It is that range of biodiversity that we must care for - the whole thing - rather than just one or two stars*."

- David Attenborough

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Summary

Understanding the depth and functioning of vast biological diversity in soils is a daunting task rife with debates and unknowns. The ecological importance of biological diversity is currently a pressing and global ecological issue. The rising demands of modern society on the Earths' resources incur an external cost on its biodiversity, including soils. Theoretically, the simplification of soil biodiversity can have consequences on the ecosystem processes soil biota support, such as productivity and nutrient retention in agricultural systems. However, the requirement to maintain high levels of biodiversity to achieve optimal functioning of ecosystems is not clear. Moreover, knowledge of the functioning of soil biodiversity is lacking due to reliance on technological advances to detect, culture, and manipulate soil organisms experimentally. The overall objective of this dissertation is to assess the potential impact of soil biodiversity loss on ecosystem functioning as well as attempt to unravel some the mechanisms by which a more diverse soil community can improve ecosystem functioning.

Since, the ability for greater biodiversity to improve ecosystem functioning requires the coexistence among species, I first address whether plant coexistence (between a grass and legume) is differentially influenced by the identity and diversity of species belonging to an influential group of symbiotic root endophytes: the arbuscular mycorrhizal fungi (Chapter 1). However, the productivity and functioning of a species community is not only determined by their biotic interactions, but also by the abiotic environmental conditions. Considering this, the experiment was replicated in both a high and low fertile substrate. In all cases, the presence of a fungal symbiont improved the coexistence between the grass and the legume by improving the competitive ability of the legume. However, whether the identity of a specific fungus present, or whether a community of these fungi improved the competitiveness of the legume best depended upon the productivity of the substrate.

To further assess the mechanisms behind the observed effects of a more fungal rich community on plant productivity, I examine the abundance and contribution of individual fungal species to plant productivity within the same substrates along a gradient of increasing fungal richness (Chapter 2). By partitioning out competitive and complementary effects among fungi on plant productivity, I reveal that fungal species richness-plant productivity relationship can be driven by both the presence of the most influential species as well as by the fungal community functioning in concert, depending on the productivity of the substrate.

The communal functioning of a diverse community of these fungi not only requires their coexistence, but also functional diversity, such that each fungal species is able to provide an additional benefit to the productivity of the plant community. This could result from differences in host or substrate preference where fungi differ in their ability to extract resources from different substrates to benefit a particular host (Chapter 3). Although differences in host and substrate preferences occurred, there was little convincing evidence to support host and substrate niche partitioning as a mechanism by which this fungal community functions to support plant community productivity.

Considering arbuscular mycorrhizal fungi are only one trophic – functional guild of organisms within highly biodiverse soils, it is important to address biodiversity loss from soils in a holistic manner. Using self-contained microcosms to maintain degraded soil biodiversity along a gradient, I demonstrate in a repeatable manner that soil biodiversity loss has negative consequences on aboveground and belowground ecosystem processes (Chapter 4). Reductions in soil biodiversity caused a decline in plant biodiversity resulting in a grass dominated plant community with varying effects on net productivity. Decomposition of organic matter, the turnover of nitrogen, and the retention of nutrients declined with soil biodiversity loss emphasizing soil biodiversity as a support system for achieving optimal levels of multiple ecosystem functions.

The majority of the work illustrates the influence of soil biota in shaping plant community and ecosystem characteristics, however, these highly diverse soil communities may not only drive ecosystem processes, but also the evolution of terrestrial plants. In an attempt to explore novel directions by which soil biota and their diversity shape ecosystems, I assess the influence of native and non-native root associated microbial communities on plant performance between two generations in two populations of *Trifolium pratense* that differ in genetic traits (Chapter 5). The populations differed markedly. Progeny of the 4x pedigree was better suited to benefiting from the soil community conditioned by their parental population while the progeny of the 2x pedigree also acquired unique soil communities, but responded the same as the parental population. This suggests an evolutionary link between soil communities and their associated plants that may drive genetic diversity and ecological differentiation in plant populations.

This research demonstrates the variety of key ecological roles of soil biodiversity: 1. It demonstrates that species richness within a trophic guild can be

important for plant productivity, 2. That soil biodiversity degradation can reduce ecosystem functioning, and 3. That there are co-evolutionary links between plants and their associated soil communities. However, a comprehensive understanding of the functioning of soil biodiversity in natural settings remains a major challenge for the future. With increasing technological advances improving both empirical and analytical aspects of soil ecology, exciting discoveries are yet to be made for comprehending the natural environment and the role soil biodiversity holds in supporting the ecosystems human societies depend upon.

Zusammenfassung

Die Tiefe und die Funktion der gewaltigen biologischen Diversität in Böden zu verstehen ist eine abschreckende Aufgabe voller Debatten und Unbekanntem. Die ökologische Relevanz dieser unübertroffenen Diversität ist eine drängende und globale ökologische Frage. Die steigenden Ansprüche der modernen Gesellschaften an die Resourcen der Erde laden zusätzliche Kosten auf ihre Diversität, einschliesslich der Böden. Die Reduzierung der Bodenbiodiversität wird wahrscheinlich Auswirkungen auf die von Bodenlebewesen unterstützten Ökosystemprozesse haben, welche notwendig sind, die Kreisläufe von Ressourcen sowie das Leben auf der Erde aufrecht zu erhalten. Es ist jedoch unklar, was die Bedingungen für die Erhaltung hoher Biodiversitätslevel sind, um ein optimales Funktionieren von Ökosystemen zu gewährleisten. Darüber hinaus fehlt das Wissen über die Funktion der Bodenbiodiversität aufgrund der Abhängigkeit von technologischen Fortschritten um Bodenorganismen experimentell detektieren, kultivieren und manipulieren zu können.

Das übergeordnete Ziel dieser Dissertation ist es, die potentiellen Auswirkungen vom Verlust von Bodenbiodiversität auf das Funktionieren von Ökosystemen ab zu schätzen, sowie zu versuchen, einige der Mechanismen auf zu decken durch die eine diversere Bodengemeinschaft die Ökosystemfunktionen verbessern kann.

Da die Koexistenz verschiedener Pflanzenarten notwendig ist, um die Ökosystemenfunktionen durch grössere Biodiversität zu steigern, werde ich zunächst der Frage nachgehen, ob Pflanzenkoexistenz (zwischen einem Gras und einer Leguminose) durch die Identität und Diversität von Arten einer wichtigen Gruppe symbiotischer Wurzelendophyten, den Arbuskulären Mykorrhizapilzen, unterschiedlich beeinflusst wird (Kapitel 1). Die Produktivität und Funktion einer Artengemeinschaft wird jedoch nicht nur durch biotische Interaktionen, sondern auch durch abiotische Umweltbedingungen beeinflusst. Um dem gerecht zu werden, wurde das Experiment in jeweils einem Substrat hoher und geringer Fruchtbarkeit wiederholt. In allen Fällen wurde die Koexistenz zwischen Gras und Leguminose durch die Präsenz eines Pilz-Symbionten verbessert, indem die Wettbewerbsfähigkeit der Leguminose gesteigert wurde. Jedoch war es von der Fruchtbarkeit des Substrates abhängig, ob die Identität eines bestimmten Pilzes, oder ob eine Gemeinschaft dieser Pilze die Wettbewerbsfähigkeit der Leguminose steigerte.

Um die Mechanismen hinter den beobachteten Effekten einer pilzreicheren Gemeinschaft auf die Pflanzenproduktivität zu erschliessen, untersuche ich die Abundanz und den Beitrag individueller Pilzarten zur Pflanzenproduktivität innerhalb desselben Substrates entlang eines Gradienten steigender Pilzvielfalt (Kapitel 2). Indem ich Wettbewerbs- und Ergänzungseffekte von Pilzen auf die Pflanzenproduktivität voneinander abgrenze, enthülle ich, dass das Pilzartenreichtum- Pflanzenproduktivitäts-Verhältnis von der Präsenz der einflussreichsten Art, sowie auch von der Pilzartengemeinschaft in Zusammenarbeit bestimmt werden kann, abhängig von der Fruchtbarkeit des Substrates.
Das gemeinschaftliche Funktionieren einer diversen Gesellschaft dieser Pilze erfordert nicht nur ihre Koexistenz, sondern auch funktionale Diversität, so dass jede Pilzart fähig ist, einen zusätzlichen Beitrag zur Produktivität der Pflanzengesellschaft zu leisten.

Dies könnte aus unterschiedlichen Wirts- oder Substratspräferenzen, wo Pilze sich in ihrer Fähigkeit Ressourcen aus unterschiedlichen Substraten einem bestimmten Wirt zur Verfügung zu stellen unterscheiden, resultieren (Kapitel 3). Obwohl Unterschiede in Wirts- und Substratspräferenzen auftraten, gab es wenig überzeugende Beweise dafür, Wirts- und Substrats- Nichenaufteilung als einen Mechanismus herauszustellen, durch den die Pilzgemeinschaft die Produktivität der Pflanzengemeinschaft unterstützt.

Wenn man beachtet, dass Arbuskuläre Mykorrhizapilze nur eine trophische und funktionale Gilde von Organismen innerhalb von hoch biodiversen Böden darstellen, ist es wichtig Biodiversitätsverlust in Böden auch einer holistischen Betrachtung zu unterziehen.

Indem ich sich selbst erhaltende Mikrokosmen benutze um degeradierte Bodenbiodiversität entlang eines Gradienten aufrecht zu erhalten, zeige ich in wiederholbarer Weise, dass Biodiversitätsverlust negative Auswirkungen auf Ober- und Unterirdische Ökosystemprozesse hat (Kapitel 4). Eine Reduzierung der Bodenbiodiversität verursachte einen Rückgang der Pflanzendiversität, was in einer Gras-dominierten Pflanzengemeinschaft mit variierenden Effekten auf die netto Produktivität resultierte.

Die Dekomposition organischen Materials, die Stickstoffumwandlung und die Zurückhaltung von Nährstoffen wurden mit dem Verlust an Bodenbiodiversität reduziert, was die unterstützende Rolle der Bodenbiodiversität um optimale Level an vielfältigen Ökosytemfunktionen zu erreichen, herausstellt.

Ein Grossteil der Arbeiten zeigt den Einfluss von Bodenlebewesen auf die Gestalt von Pflanzengemeinschaften und Ökosystemcharakteristiken auf, doch diese hoch diversen Bodengemeinschaften könnten nicht nur Ökosystemprozesse, sondern auch die Evolution der Landpflanzen steuern. In einem Versuch, neue Wege zu erschliessen, wie Bodenorganismen und ihre Diversität Ökosysteme formen, untersuche ich den Einfluss heimischer und nicht-heimischer Wurzelassoziierter mikrobieller Gemeinschaften auf das Pflanzenwachstum in je zwei Generationen und Populationen von *Trifolium pratense*, welche sich in ihren genetischen Eigenschaften unterscheiden (Kapitel 5). Die Populationen unterschieden sich deutlich. Nachkommen des 4x Stammes waren besser geeignet, von der von der Elternpopulation geprägten Bodengemeinschaft zu profitieren, während die Nachkommen des 2x Stammes auch einzigartige Bodengemeinschaften ausbildeten, aber sich gleich der Elternpopulation verhielten. Dies deutet auf eine evolutionäre Verknüpfung zwischen Bodengemeinschaften und ihren assoziierten Pflanzen hin, die genetische Diversität und ökologische Differenzierung in Pflanzengemeinschaften steuern könnte.

Diese Forschung zeigt die Vielzahl ökologischer Schlüsselrollen der Bodenbiodiversität- von Artenreichtum innerhalb einer trophischen Gilde und allgemeinem Bodenbiodiversitätsverlust für Ökosystemfunktionen, zu der möglichen koevolutionären Verknüpfung zwischen Pflanzen und ihren assoziierten Bodengemeinschaften- auf. Ein umfassendes Verständnis der Funktionen von Bodenbiodiversität im natürlichen Umfeld über zeitliche und umweltbedingte Variationen hinweg bleibt jedoch unerreicht. Mit fortschreitenden technischen Entwicklungen in empirischen und analytischen Aspekten der Bodenökologie, gibt es noch viele aufregende Entdeckungen zu machen um die natürliche Umgebung und die Rolle der Bodenbiodiversität für die Unterstützung der Ökosysteme zu begreifen, von denen menschliche Gesellschaften abhängen.

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Curriculum Vitae

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EDUCATION

B.Sc. 2005. Environmental Science. University of Guelph, Canada.
Major: Ecology
Minor: International Development

M.Sc. 2007. Integrative Biology (Botany). University of Guelph, Canada.
Thesis: Growth response of two co-occurring conifer tree species to native mycorrhiza, soil quality and seed source elevation.

Ph.D. 2012. Institute of Environmental Sciences, University of Zürich, Switzerland.
Thesis: Ecological Roles of Soil Biodiversity.

SOCIETIES (PAST AND PRESENT)

International Mycological Association - *MSA*
International Mycorrhiza Society - *IMS*
Canadian Botanical Association - *CBA*
Canadian Society for Ecology and Evolution - *CSEE*
European Plant Science Organisation – *EPSO*
International Symbiosis Society - ISS

JOURNAL AFFILIATIONS

Faculty of 1000
Frontiers In Functional Plant Ecology
Reviewer for: *Ecology Letters*; *Journal of Ecology*; *New Phytologist*; *Plant and Soil*; *American Journal of Botany*; *Acta Physiologiae Plantarum*; *PLoS1*

STUDENTS CO-SUPERVISED

Samuel Ayesu. 2010. MSc Thesis "*The importance of soil biodiversity for plant productivity in a heterogeneous soil environment*" Vrije Universiteit Amsterdam – Agroscope Reckenholz-Tänikon Research Station with Prof. Marcel van der Heijden.

Chirstoph Barendregt. 2012. MSc Thesis "*Temporal development of bidirectional biodiversity effects in plant-mycorrhizal communities*" University of Zürich – Agroscope Reckenholz-Tänikon Research Station with Prof. Marcel van der Heijden.

PUBLICATIONS

Wagg, C., Boller, B., Schneider, S., Widmer, F. and van der Heijden, M.G.A. (submitted) Plant-soil feedback as a potential evolutionary mechanism.

Thiels-Bruhn, S., Bloem, J.,de Vries F.T., Kalbitz, K., van der Heijden, M.G.A. and ***Wagg, C.*** (2012) Linking soil biodiversity and agricultural soil management. *Current Opinion in Environmental Sustainability* (*in press*).

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BOOK CHAPTERS

Wagg C, Veiga R, van der Heijden MGA. (*pending*). Antagonism and facilitation in mycorrhizal networks. *In* Mycorrhizal Networks. *eds*. Horton TR. Springer.

OTHER PUBLISHED WORKS AND CONTRIBUTIONS

Microscopy images

Peterson, R.L., Massicotte, H.B., Melville, L.H. and Phillips, F. (2006) Mycorrhizas: anatomy and cell biology images. NRC Research Press. Ottawa, Ontario. (CD-ROM)

Zimmerman, E. and Peterson, R.L. (2007) Effect of a dark septate fungal endophyte on seed germination and protocorm development in a terrestrial orchid. *Symbiosis* 43:45-52.

Peterson, R.L., Peterson, C.A. and Melville, L.H. (2008) Teaching plant anatomy through creative laboratory exercises. NRC Research Press, Ottawa, Ontario.

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MSc. Thesis

Wagg, C. 2007. Growth response of two co-occurring conifer tree species to native mycorrhiza, soil quality and seed source elevation. MSc. Dissertation, University of Guelph, Canada. ISBN: 9780494365786

MEETINGS AND PRESENTATIONS

Plant Development Workshop, November 2005. Guelph, Canada.

International Conference on Mycorrhiza (ICOM). July 2006. Grenada, Spain.

Canadian Society for Ecology and Evolution (CSEE). May 2007. Toronto, Canada.
Presentation: The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal and dark septate fungi in seedlings of four members of the Pinaceae.

Plant Canada Conference (Canadian Botanical Association). June 2007. Saskatoon, Canada.
Presentation: Influences of soil fertility and mycorrhizal associations on lodgepole pine and interior hybrid spruce seedling growth.

3ème Cycle Homand en Sciences Biologiques: Polyploidization, plant fitness and trophic interactions. September 2009. Fribourg, Switzerland.

Canadian Society for Ecology and Evolution (CSEE). May 2010. Quebec, Canada.
Presentation: Belowground diversity and aboveground productivity: the role of mycorrhizal fungi.

Swiss Microbial Ecology (SME). January 2009, 2011, Engelberg Switzerland.

Annual Symposium for PhD Students of Agroscope (ASPSA) 2008, 2009, 2010.

International Mycological Congress (IMC). August 2010. Edinbourgh, UK.

Wageningen Conference on Applied Soil Science: Soil Science in a changing world. September 2011, Wageningen, Netherlands.
Presentation: Belowground biodiversity effects support aboveground productivity.

1st internatinal conference on Evolution, July 2012. Ottawa, Canada.

AWARDS AND HONOURS

University Graduate Scholarship. 2006. (University of Guelph – Dept. Botany).

Latornell Graduate Travel Scholarship. 2006. (University of Guelph).

Lionel Cinq-Mars Award. 2007. Honourable mention for best student paper presentation. Canadian Botanical Association (Plant Canada Conference 2007).

John Macoun Travel Bursary. 2007. Canadian Botanical Association.

TEACHING

Music Teacher, 1996-1999. Ontario Conservatory of Music, London, Ontario, Canada.

Elements of Calculus I. Peer-Tutor. Winter 2003. University of Guelph, Canada.

Statistics in Political Science. Peer-Tutor. Fall 2003. University of Guelph, Canada.

Plant Anatomy. Teaching Assistant. Fall 2004. University of Guelph, Canada.

Biology I. Teaching Assistant, Fall 2005. University of Guelph, Canada.

Field Ecology. Teaching Assistant, Summer 2006 & 2007. University of Guelph, Canada and the Algonquin Park Wildlife Research Station (Ontario, Canada).